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FOREWORD

The publication of the 1954 Surgical Forum Volume marks another milestone in the history of the Surgical Forum of the American College of Surgeons. Here, in a single volume, the reader will find the papers that were selected for a presentation at the Clinical Congress of the American College of Surgeons in Atlantic City in November, 1954. They provide written testimony of the direction in which surgical effort in investigation is going in this country.

If anyone doubts that American surgery will continue to go forward in the future, he need only scan this volume. If he wishes to keep abreast of modern developments in surgery, he must read this volume.

With growing insight, young surgeons and men in training in surgery and in the surgical specialties are attesting to the fact that the modern surgeon is more than a simple technician. They are demonstrating that surgeons can also have a deep understanding of biochemistry and physiology and biophysics. The early isolation of surgery from its sister sciences is disappearing, and the horizon of the surgeon is being extended.

It is hoped that the papers presented in this volume will give those who read them a deep understanding of many of the biochemical and physiologic problems which now concern the surgical patient. Elliot Cutler once said that the triad upon which the competency of a hospital should be judged was the care it gave its patients, the teaching and training it provided, and the research which it did. In this volume, one finds that departments of surgery in this country are fulfilling, in large measure, the obligations which they have. Charles Mayo once said, "It took the world from the day of its creation to the time of the sixteenth century to raise a doubting Thomas of sufficient mental strength and courage to state that questions were not answered by authority, but by experiment."

The American College of Surgeons proudly presents this volume to all those who are interested in advances in surgical knowledge. In it will be found cornerstones upon which the future will be built.

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HEART AND GREAT VESSELS

INTRODUCTION

HARRIS B. SHUMACKER, JR.

In addition to the papers presented at the Forum sessions, other contributions concerning the heart and great blood vessels were brought forth in the postgraduate course, in the Ciné Clinics, and in a number of panel discussions. From all of these excellent presentations it is evident that important recent advances have been made in this area.

Experience with excisional therapy of thoracic and abdominal aneurysms has increased and it is now apparent that such measures are generally curative and can be carried out with a reasonable mortality. Observations concerning the use of homografts have been quite satisfactory. Various methods for the sterilization of such grafts are reported by Szilagyi and his associates, Harkins and his colleagues, and MaCris, Sloan and Orebaugh. From a number of Forum presentations, as well as from material given in the postgraduate course, it is clear that pliable plastic prostheses serve satisfactorily for aortic replacement. It is evident from the discussion of these papers that most surgeons have no hesitation in using such plastic grafts for aortic and aortic bifurcation replacement in man.

Some of the studies included in this volume have expanded our knowledge of the functional disturbances associated with certain cardiac disorders. As examples may be mentioned the work of Ferguson and his associates on experimentally produced patent ductus type shunts, the study of Bowes and his colleagues on experimentally produced abnormal pulmonary venous drainage, and the studies concerning valvular dysfunction by Meckstroth and Klassen, Himmelstein and his associates, and Hurwitt, Hoffert and Ferreira. A number of experimental operative procedures of potential clinical applicability were presented. Among these are the studies by Glenn and Turk of a vascularized graft passed through the mitral ring, the direct operations upon the mitral valve by Shumway and Lewis, the plastic operation upon the atrial septum proposed by Albert as a possible means of correcting the functional derangement in cases of complete transposition of the great vessels, and the total by-passing of the right ventricle by Warden, DeWall and Varco.

A great deal of work aimed at the development of a safe method for open surgery within the cardiac chambers was described. Gibbon reported that his artificial heart-lung apparatus had been modified and improved and that recent animal experimental studies gave him encouragement to proceed once again with the clinical application of this machine for total cardiac and pulmonary by-pass One panel discussion, several papers in the postgraduate course and a number of studies presented in the Forum sessions dealt with the use of hypothermia. Conflicting views concerning the effect of carbon dioxide upon the incidence of ventricular fibrillation were presented by Niazi and Lewis on the one hand and Swan and his group on the other. It is important that the correct solution to this problem be firmly established. Swan reported a reduction in the incidence of ven-

SHOCK AND WOUNDS, PLASTIC ANATOMIC CAST

EXPERIENCE WITH THE BJORK-CRAFOORD OPERATION FOR CLOSURE OF ATRIAL SEPTAL DEFECT

JAMES L. SOUTHWORTH AND C. HARWELL DABBS*

Of the numerous experimental and clinical methods described for closure of atrial septal defects, that of Bjork and Crafoord[1] (which they credit to a suggestion of T. Sondergaard) was selected for trial. In this operation the two atria are dissected apart and a suture is passed and tied in such a way that the dissected atrial wall is brought down to form a septum without interfering with the atrioventricular valves or the orifices of the veins. As stated by Bjork and Crafoord, it appears that this operation should be suitable for any type of defect including those of the ostium primum type.

ANIMAL EXPERIMENTS

To test this assumption, ten dogs were subjected to operation. The animals, all mongrels, were anesthetized with pentobarbital and maintained on intermittent positive pressure respiration. The right chest was opened through the fourth or fifth interspace, the heart exposed, and the pericardium incised. At first the pericardium was opened anterior to the phrenic nerve, but we came to prefer opening it posteriorly because the remaining posterior rim of pericardium offers a convenient grasping point to begin the interatrial dissection. A Gross well was attached to the right atrium and atrial septal defects were created. With a finger in the right atrium as a guide, dissection was started between the superior vena cava and the superior pulmonary vein and carried down to the rim of the atrial defect. Twice the dissection was carried too deeply and we learned to recognize the appearance of blood within the chamber retained only by endocardium; once the atrium was accidentally opened during the dissection, but no difficulty was encountered in sewing the defect and the operation was completed satisfactorily. We found it advisable to open the plane of separation quite deep superiorly in order to be able to see the aorta down to the origin of the coronary artery; this maneuver facilitates placing the suture without needling the aorta and facilitates starting the suture at the optimum point—either into the inferior rim of a high defect or directly into the interventricular septum between the atrioventricular valves in the case of a low defect.

This dissection and the subsequent subendocardial passage of a suture were tolerated well by all animals. The suture was tied by an assistant while the operator palpated the interior of the heart and made certain that no important structures were compromised. Except for one animal sacrificed acutely for the purpose of photography, all animals tolerated the entire operation uneventfully and made good recoveries. When sacrificed at intervals of one to four weeks postoperatively, the defects were held closed by the suture (early) or had healed.

CLINICAL TRIAL

We were impressed by the relative technical simplicity of this operation and by the absence of bad effect either during the operation or during the short follow-up period and were thus encouraged to use it on a patient.

* Knoxville, Tennessee.

tricular fibrillation during open ventriculotomy in the hypothermic state as the result of coronary perfusion with a dilute Prostigmin solution. The paper by Kirby, Jensen and Johnson adds to our information concerning defibrillation of the ventricles under hypothermic conditions. It is evident from work described during this Clinical Congress that hypothermia has provided a relatively safe method for open operations upon the pulmonary valve and the atrial septum. *It is not felt that evidence is yet at hand to* suggest that pulmonary valvular stenosis and atrial septal defects should now be attacked routinely by open operations, since they can generally be managed quite well by closed procedures not requiring interference with cardiac action

The experimental and clinical use of controlled cross circulation for open operations within the ventricle by Lillehei and his associates constitutes a daring and significant contribution It has permitted them to demonstrate that direct suture closure of ventricular septal defects is feasible. It has also shown that a markedly reduced circulation may be adequate for short open operations upon the heart. The chief factor which deters others from utilizing this method is the potential hazard to the donor. In spite of the fact that Lillehei and his group feel confident that controlled cross circulation can be carried out without real risk to the donor, many surgeons fear that the method does subject the donor to danger Small as this risk may be, they feel it makes the use of this principle inadvisable.

and is taking classes to learn stenography. As far as can be determined she has no symptoms referable to the cardiovascular system. Catheterization studies,[4] however, show persistence of a left to right shunt. Oxygen saturations are as follows: superior vena cava, 50 per cent; right atrium, 79 to 89 per cent, depending upon position of the catheter tip, right ventricle, 86 per cent, pulmonary artery, about 90 per cent. The mean pressures are: right atrium, 5 mm. Hg, right ventricle, 35 mm.; pulmonary artery, 60 mm.

CONCLUSIONS

1. From this limited experience, the Bjork-Crafoord operation appears to be a relatively simple operation for closure of atrial septal defects, particularly of the ostium primum type. Its simplicity is relative in the sense that no elaborate apparatus or tedious preparatory methods are required.

2. The operation is well tolerated. If closure of an atrial defect should cause deterioration of the circulation, the closure would be readily reversible.

3. We found preliminary animal work of great help in preparing for operation on the patient.

4. The ultimate place of this procedure in treatment of atrial septal defect in patients remains to be determined.

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THE IMPLANTATION OF A VASCULARIZED GRAFT IN THE CHAMBERS OF THE HEART*

An Experimental Approach to the Correction of Valvular Insufficiency by Means of a Vertically Suspended Graft

WILLIAM W. L. GLENN, L. NEWTON TURK III, AND
THOMAS O. GENTSCH

In the course of more than 125 operations for mitral stenosis we have been confronted with a major degree of mitral insufficiency in four patients. In each of these four patients the area of valvular deficiency could be effectively plugged by the surgeon's index finger. We therefore decided to at-

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This patient (previously reported briefly[2]) is a 32-year-old white female who had a murmur since childhood. No clear-cut history of rheumatic fever can be elicited. At about age 28 she began to experience fatigue and subsequently had a number of episodes of cardiac failure Upon examination she was seen to have no cyanosis, she presented the gracile habitus mentioned by Taussig.[3] The central portion of the chest was prominent A few moist rales were heard at both bases. The heart was enlarged to the right but not to the left, and a very loud systolic murmur was heard on the left. The pulmonic second sound was accentuated, as was the mitral first sound. There was some disagreement among several observers about the existence or type of mitral valve murmur. The liver could be palpated below the costal margin, but no edema was present. Her weight was 100 pounds, temperature 98 6, pulse at rest, 90; and blood pressure 130/90.

Fluoroscopic examination of the heart disclosed increased vascularity of the lung fields, an inconspicuous aorta, a prominent pulmonary artery shadow, and enlargement of t' [illegible]
ble enlargement of the left ve [illegible]
oxygen saturations. superior [illegible]
cent, right ventricle, 92 per cent, and pulmonary artery, 92 per cent. The mean pressures were· right atrium, 6 mm Hg, right ventricle, 35 mm., and pulmonary artery, 42 mm.

At operation February 12, 1954, a Gross atrial well was attached to the greatly enlarged and thickened right atrium and a huge atrial septal defect of the ostium primum type found. Superiorly and laterally, a narrow rim of septal wall remained, but there was none elsewhere. The pulmonary veins all appeared to enter in proper relationship to the venae cavae The margins of the atrioventricular valve leaflets were a little beaded and rolled, the medial leaflet of the tricuspid valve was calcified. A light regurgitant jet could be palpated at the mitral valve. Because of the experience with animals, it was easy to dissect the atria apart insofar as the remaining interior rim permitted and to pass a suture subendocardially into the interventricular septum and to bring it out inferiorly between the inferior vena cava and the inferior pulmonary vein An assistant tied the suture while the operator palpated the defect and adjacent structures The defect was completely closed at first and held so for a time. No circulatory derangements resulted, but upon palpation it seemed as if a small hillock of redundant tissue had been created in front of the opening of the coronary sinus, and we feared that subsequent reaction might partially occlude this orifice Therefore the suture was loosened a little, leaving a defect about 4 mm in diameter, this relaxed the puckered thick atrial wall enough that there seemed to be no danger of encroachment on any orifice or important structure. There were no arrhythmias from sewing into the interventricular septum

Postoperatively the patient did well and has continued to do very well clinically to the time of this writing (October 10, 1954) She has gained to 115 pounds without edema and there have been no further episodes of congestive failure. The murmur is greatly diminished in intensity, but the pulmonic second sound remains accentuated. The right atrial shadow is less prominent by x-ray, the left atrial shadow has become enlarged, and the lung fields still appear congested The patient's appearance is greatly changed for the better, owing to filling out of her thin arms, legs, and face. She insists on doing all her own housework, including the heavy cleaning,

insufficiency. It is conceivable that this technique might be useful also to plug certain other valvular defects or defects in the cardiac septa.

TECHNIQUE

Healthy mongrel dogs were used in these experiments. Two types of vascularized grafts were employed. The first type made use of the pericardiophrenic vessels with a strip of the attached vascularized pericardium on either side The phrenic nerve was sacrificed. The second type of graft employed the freed internal mammary artery and vein with the immediately adjacent perivascular tissues dissected from the origin of the internal mammary artery to the level of the eighth rib. A segment of autogenous vein



Fig. 1



Fig 2

Fig. 1. Graft in place at time of sacrifice L.A. is above, L.V. below. Arrow points to window cut in graft to show black vinylite plastic which has been injected into the internal mammary artery

Fig. 2. After acid digestion, vinylite cast of coronary system (white) and internal mammary artery (black), which is patent to its point of insertion in left ventricle

sufficient in length to reach from the top of the left auricle to just beyond the apex of the left ventricle was sewed to a hole made in the left auricle. A piece of auricular tissue was actually removed to create a round hole to which the vein was sewed. A long needle with braided wire threaded to a detachable point[4] was passed first through the vein graft and then through the auricle and ventricle, traversing the atrioventricular channel and directed out of the wall of the left ventricle. The needle may also be passed in the reverse direction The wire was tied to the graft of tissue, and the tissue was made to invaginate the vein [illegible] ing for the vascularized pedicle of tiss[illegible] the contained vascularized pedicle wa[illegible] with sutures (Figs. 1 and 2).

tempt to construct from the patient's own tissues an effective "finger" to plug the area of deficiency in the approximation of the valve cusps.

Several different techniques have been explored for the relief of insufficiency of the mitral valve. These include those techniques designed (*a*) to narrow or constrict the atrioventricular opening[2, 3]; (*b*) to replace the loss of valve substance by a graft of tissue or a plastic prosthesis below, above or as a part of the valve[5, 6, 7, 8, 10], and (*c*) to sew the valve leaflets together substituting a moderate degree of stenosis for a severe degree of insufficiency.[1]

We have approached the problem of the surgical treatment of insufficiency of the atrioventricular valves with the following considerations in mind

1. In chronic inactive endocarditis where mitral insufficiency has resulted, the degree of insufficiency is usually static and can be expected to remain so, provided reinfection does not occur or calcium deposits along the leaflets do not decrease the size of the deficient area On the other hand, the area of insufficiency in acute myocardial dilatation which is due to rheumatic disease or other causes is not to be considered a surgically correctable lesion

2 The area of insufficiency of the atrioventricular valve represents an absolute loss of valve area which must be replaced or plugged to prevent leakage of blood back into the auricle during ventricular systole.

3 To withstand the constant attrition of the atrioventricular valve the filler for the deficient area should be incapable of injury by the constant movement of the valve cusps, or else responsive to such trauma by prompt repair Our experience with closure of interventricular septal defects using a plastic sheet has suggested to us that plastic materials within the heart are not always well tolerated, and we have preferred, therefore, not to use them in the repair of the atrioventricular valves Also, our experience with avascular tissues placed within the chambers of the heart, as reported before this forum one year ago, has shown that a high percentage of these grafts become infected, show degenerative changes and fragment We have, therefore, considered it especially important to construct a prosthesis of tissue distinguished particularly by a more than adequate blood supply. To assure a smooth surface to this graft we have placed it inside an inverted autogenous vein segment.

4. A number of attempts have been made to construct from tissue or plastic a "functioning" atrioventricular valve. Generally, these attempts have been discouraging,[5, 10] although others[9, 11] have been more encouraging concerning the function of the artificial prosthesis designed by them. We have preferred not to rely on the "function" of a graft except in the performance of a passive role We would therefore place the graft *vertically* through the deficient area so that the valve cusps may approximate around it.

The concept in theory is of a well vascularized endothelial covered graft extending *vertically* through the atrial wall, across the atrial chamber, through the atrioventricular valve where the cusps may approximate the vertical walls of the graft, across the long axis of the ventricular chamber and out of the ventricular wall at a point designed to hold the graft in the deficient area of the atrioventricular valve. The technique might be used to plug a deficient area in either of the atrioventricular valves. The same principle might possibly be used in the case of severe aortic or pulmonary

Table 1. Fate of an Intracardiac Vascularized Graft—(Continued)

EXPERIMENT NO.	DURATION OF SURVIVAL, DAYS	CAUSE OF DEATH	PATENCY OF ARTERY	ADHESION OF GRAFT TO VALVE	REMARKS
17	245	Sacrificed	+	+	Densely adherent to valve Small anastomoses with coronary system
18	239†	Sacrificed	+	0	Slight thickening of graft at point of contact with valve
19	133	Sacrificed	+	0	Excellent graft Small anastomosis with coronary system noted
20	233	Sacrificed	+	+	Graft short, pressing anterior cusp of valve against myocardium Adherent
21	70	Sacrificed	+	0	Small area of granulation at junction of graft with left auricle
22	92	Sacrificed	+	0	Several small verrucous growths on cusps at point of contact with graft Small anastomoses with coronary system
23	89	Sacrificed	+	+	Graft short and adherent to anterior cusp Artery narrowed below point of entry into left auricle

* IMA and V: Internal mammary artery and vein enclosed in segment of inverted autogenous vein was used in this and subsequent experiments

† In Figures 1 and 2 this is shown as 249 days, but 239 days is correct.

The results in the 23 experiments are listed in Table 1. In only four experiments was the major arterial supply to the graft found to be thrombosed at the time of sacrifice. These were all early experiments. In seven experiments there were adhesions between the graft and the valve cusps. In most of these the graft had been brought out of the left ventricle just below the valve. The graft in these experiments pressed directly on the valve cusp, which may have encouraged adhesions between the two structures. In several experiments small anastomoses with the coronary vessels were demonstrated by vinylite plastic injection[9] of the coronary and internal mammary vessels. In the last three experiments an attempt was made to encourage these anastomoses by pulling the internal mammary artery free of the vein sheath after it emerged from the wall of the left ventricle, and burying its open end directly into the myocardium.[11] This did not appear to encourage anastomoses with the coronary system, but the artery may have been under tension in these experiments.

Except in the experiments where the valve was adherent to the graft or in the one experiment where death occurred as a result of subacute bacterial endocarditis, changes in the valve leaflets consisted of slight thickening of the edge of the valve cusp at the point where they met the graft and, in two experiments, a small amount of granulation tissue on the graft or valve edge or both.

No murmurs were ever heard in any of these animals, and no unusual

RESULTS

A total of 23 experiments have been performed. All animals that have not died have been sacrificed. There were three immediate deaths related to the technique In two of these we perforated a major coronary branch with the long needle, an accident which should be easily avoided The third animal died of a bilateral pneumothorax soon after operation One other animal died of a chest wound infection and dehiscence eight days after operation, and another animal died of subacute bacterial endocarditis five weeks after operation. All animals received penicillin and streptomycin for four to ten days postoperatively.

Table 1 Fate of an Intracardiac Vascularized Graft

EXPERIMENT NO	DURATION OF SURVIVAL, DAYS	CAUSE OF DEATH	PATENCY OF ARTERY	ADHESION OF GRAFT TO VALVE	REMARKS
1	222	Sacrificed	0	0	Pericardiophrenic vessels No vein sheath Free of adhesions to valves
2	209	Sacrificed	0	0	IMA and V* no adhesion of graft to valve edges Graft shrunken and fibrous
3	309	Sacrificed	0	+	Small granulomatous lesions on graft and adjacent myocardium Valve cusps free of adhesions to graft except for one small area
4	8	Infection	+	0	Wound dehisced
5	201	Sacrificed	+	0	Excellent graft Widely patent artery to apex
6	299	Sacrificed	0	+	Adhesion to anterior leaflet Short, shrunken graft First attempt by second operator
7	0	Pneumothorax	–	–	Found dead two hrs postoperatively Bilateral pneumothorax
8	190	Sacrificed	+	0	Small granuloma on graft just below entry into left auricle
9	68	Distemper Sacrificed	+	0	Graft in excellent condition, soft, pliable
10	172	Sacrificed	+	0	Small area of calcification of graft at point of contact with valve cusp
11	165	Sacrificed	+	0	Excellent graft
12	261	Sacrificed	+	0	Small anastomosis of graft artery to coronary system
13	34	Endocarditis	+	+	Gross ulceration of valve and graft at point of contact
14	154	Sacrificed	+	+	Thin adhesion of graft to cusp, suggesting that graft may have pierced cusp
15	0	V F	–	–	Major coronary artery pierced by needle, ventricular fibrillation
16	0	V F	–	–	Same as No 15

to avoid predisposing factors to clot formation within the chambers of the left heart.

4. On the basis of these experiments the cautious application of this technique to severely incapacitated patients with mitral insufficiency seems justified. Our intention is to use the pericardiophrenic vessels in the human with the adjacent pericardium. We may incorporate a length of the internal mammary artery and vein as well. The whole will be invaginated through an autogenous vein graft. *It is hoped that we can tailor the plug to fit the* deficient area sufficiently well to control the majority of the leakage. We do not expect to stop the leakage altogether, but this is probably not essential.*

SUMMARY

A technique is described whereby a vascularized pedicle of tissue is passed *vertically* through the chambers of the left side of the heart and between the cusps of the atrioventricular valve. Twenty-three experiments were performed, and the fate of the trans-chamber graft is described in each.

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* After this paper was submitted for publication but before it was presented at Atlantic City on November 15, 1954, we performed experiments with a *vertically* suspended graft of molded plastic sponge (Ivalon).[7] Where used alone there was a delay in covering of the sponge with tissue at the point of contact with the mitral valve and there was also evidence of trauma to the valve cusps at this point. These objectionable features were not observed when the sponge was covered with a segment of inverted autogenous vein The first clinical trial of this technique has been made in a patient with severe mitral insufficiency and will be reported in detail in the near future.

electrocardiographic findings were observed except for a picture of pericarditis in two animals following operation

COMMENT

We made no attempt to produce or correct mitral insufficiency in these animals The lesion produced experimentally would not, we believe, be similar to that seen in the human Our interest lay in determining (1) if a pedicle graft of vascularized tissue could be found and placed in the chambers of the heart, (2) the fate of this tissue imbedded over a prolonged period, (3) the reaction of the intracardiac structures, particularly of the valves, to repeated direct contact with this tissue, and (4) the placement of this graft in several parts of the atrioventricular orifice to guide us in the proper insertion of such a graft under a variety of circumstances which might be encountered in the human.

We believe from the results of these experiments that the following facts and conjectures can be considered.

1. A graft composed of the internal mammary artery and vein invaginated in a sheath composed of an autogenous vein segment can be inserted across the left auricle, through the atrioventricular valve and through the chamber of the left ventricle and out of the wall of the left ventricle, where it is fixed to the outer wall by several suture ligatures. It is not surprising that the internal mammary artery, enclosed as it is in the vein graft, only rarely establishes anastomoses with branches of the coronary arteries It was surprising, at least to us, that the internal mammary artery remained patent in every experiment after the initial trials (Exps. 1, 2, 3 and 6). This may depend to some extent on the presence of adequate venous drainage. We do not know that the vein always remains patent, as in most of these experiments the venous drainage of the graft was not investigated.

2. The positions of entry of the graft in the auricle and of emergence of the graft from the ventricle determine the alignment of the graft in the valve area and determined to a large extent whether the graft remained free of adhesion to the valves. In the human it is hoped that the graft can be used to fill the "loss" of tissue. If the deficient area lies closer to the anterior or posterior wall of the heart, the graft can be so directed as to emerge from the ventricle at a point on the appropriate wall short of the apex. Adhesion of the graft to the valve would be expected to occur, but so long as it performed its "function" as a plug to the deficient area, the adhesion to the valve cusp would probably be of no serious consequence If the deficient area was in the center of the valve area, the graft could be directed to or near the apex, with the hope that it would remain free of adhesion to the valve cusps which would close around it.

3. Several avoidable technical errors occurred in these experiments, and two serious complications directly related to the operative procedure were observed. Subacute bacterial endocarditis developed in one animal (Exp. 13) and a transient hemifacial weakness was noted immediately postoperatively in another animal (Exp. 9), possibly related to a cerebral embolus. The endocarditis occurred five weeks postoperatively and might have been effectively treated had we suspected it earlier. The embolus to the brain during operation is a possible and constant hazard of operative procedures performed on the functioning left heart. Every precaution must be taken

sulted, this was well tolerated. Animals sacrificed at two months showed scarring with calcific deposits in the region of the suture.

Incision of the aortic leaflet of the mitral valve with subsequent suture was less well tolerated because of frequent, fatal regurgitation. In some animals the sutures had actually torn out of the leaflet substance. Normal leaflet, in general, withstood suturing poorly.

Compressed Ivalon sponge was next used to replace a window of tissue excised from the aortic leaflet. Three of 6 animals were operative deaths from massive insufficiency of the mitral valve. There were no long term survivors. In most of the dogs the prosthesis had become loose and, therefore, was no longer effective against the regurgitant jet.

In a pilot study, left ventriculotomy was carried out in 6 consecutive experiments without a death. Since shortening of the chordae tendineae is often a part of the pathologic anatomy of mitral insufficiency, an attempt

Table 1. Results of Detachment of the Posterior Rim of the Mitral Valve

NO.	LENGTH OF S[illegible]	[illegible]	[illegible]
1	1 month		
2	2 month	[illegible]	Well healed. Minimal stenosis by scarring
3	Op. death	Hemorrhage	Coronary sinus opened
4	Op death	Ventricle perforated	Dissected too far posteriorly
5	3 weeks	Atelectasis and pneumonitis	No thrombus, healing under way
6	6 weeks	Sacrificed	Complete healing
7	2 months	Sacrificed	Healed Some calcific deposit on scar
8	2 months	Sacrificed	Well healed
9	2 months	Sacrificed	Well healed
10	Op. death	Hemorrhage	———
11	2 days	Atelectasis and pneumonitis	Thrombus on line of incision
12	1 week	Atelectasis and pneumonitis	No thrombus or healing

was made to excise either papillary muscle with the chordae attached and to suture that divot nearer the atrioventricular ring. There were 8 operative [illegible]

[illegible] papillary muscle extends chordae to both cusps of the mitral valve, and while the subtended leaflet at one point may have been mobile, in another area there was shortening of the chordae and consequent insufficiency.

DETACHMENT OF THE POSTERIOR RIM

Another method of increasing the mobility of the mitral valve is detachment from the posterior ring by an incision just above the junction between leaflet and mitral ring. Dissection downward into the [illegible]

[illegible] was done. Three of 12 dogs

EXPERIMENTAL SURGERY OF THE MITRAL VALVE UNDER DIRECT VISION USING HYPOTHERMIA*

NORMAN E. SHUMWAY AND F. JOHN LEWIS

Although stenosis of the mitral valve is well treated by commissurotomy, mitral insufficiency remains a difficult problem. Closed techniques adequate for mitral stenosis have failed to supply the answer to regurgitation. For that reason this study of direct vision methods was undertaken.

Preliminary investigations indicated that direct vision surgery of the mitral valve under hypothermia was feasible. Finding a suitable operation for mitral insufficiency, however, posed real difficulty. The failure of closed techniques to satisfy completely this need can be understood from the problems encountered even under direct vision.

METHOD

Adult mongrel dogs were used without pre-anesthetic medication or pre- [illegible] antibiotics Intravenous sodium pentobarbital, 13 mg per pound, [illegible] with an electronic respirator [illegible] *immersion at 4 to 8°* [illegible] ion constituted the method of rewarming [illegible] old water when the rectal temperature registered 28° C. There was customarily a drift of 2 to 4° below *that level. Under aseptic* precautions an incision *was made in the* fifth left intercostal space. Control of the heart *is more* complete through the fifth intercostal space than the fourth. With the pericardium *widely* opened complete inflow *occlusion* was effected by means of a single umbilical tape passed through the transverse *sinus and inferiorly around* the venae *cavae and* pulmonary veins After the inflow tourniquet was made secure *and the heart was fibrillated by an electric shock*, the left atrium *was* incised from the tip of the auricular appendage to the entrance of the pulmonary *veins. The operation of choice* was then *performed* The cardiac incision was closed with a *non-crushing clamp after* the heart was filled with normal saline *Respirations were resumed* and *defibrillation was accomplished* by epinephrine, massage, and electric shock, in that order The cardiac *incision* was sutured, *and the clamp removed*, however, *the pericardium* was not reapproximated

With minor variation this was the technique used in all operations except those in which an attempt was made *to* transplant a papillary muscle closer to the atrioventricular ring. This procedure was carried out through a *long* left ventriculotomy *midway* between the papillary muscles

RESULTS OF PRELIMINARY INVESTIGATIONS

At the outset, suture methods were evaluated by making a pleat in the posterior cusp of the *mitral* valve with 3-0 silk. When no insufficiency re-

* [illegible] University of Minnesota Medical School, Minne- [illegible] National Heart [illegible] the University of [illegible]

be limited. Prosthetic baffles appear to work better than the various autogenous flutter valves.[5,6]

Suturing the left auricular appendage either completely or as a pedicle against the ventricular wall undoubtedly developed from closed invagination techniques for interatrial septal defects.[7,8] Early relief of mitral insufficiency by the method of complete invagination has been reported clinically; however, late results were discouraging.[9] Shrinkage of pedunculated atrial flaps has been noted experimentally, but the degree of shrinkage was not considered to be as hopeless as that of other autogenous slings and flaps.[10]

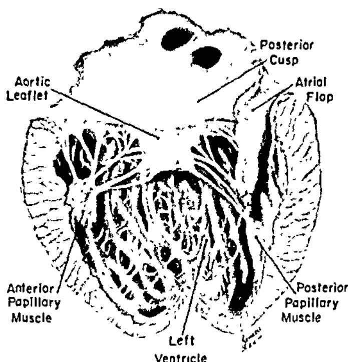


Fig. 2. Suture of atrial flap to papillary muscle.

Under direct vision it was relatively simple to suture pedicles of left auricular appendage and atrium to either papillary muscle depending upon which direction was chosen for the pedicle base. Table 2 shows the experience with this procedure, and Figure 2 is a drawing of the operation.

CONCLUSIONS

1. Even under direct vision, experimental operations to correct mitral insufficiency are difficult.

2. Suture of normal leaflet tissue is poorly tolerated. Suture of leaflet tissue scarred by rheumatic disease may be quite another thing. Suture of the mitral ring above the leaflet is readily done under direct vision and hypothermia.

3. Pedicle flaps of left auricle and atrium sutured to either papillary muscle through the mitral valve undergo late shrinkage and appear ineffective.

4. The use of a papillary muscle to anchor prosthetic material hinged at

were operative deaths. Table 1 shows the fate of the survivors. When the incision and dissection were extended to include the posterolateral commissure, a small degree of mitral stenosis was effected by the cicatricial

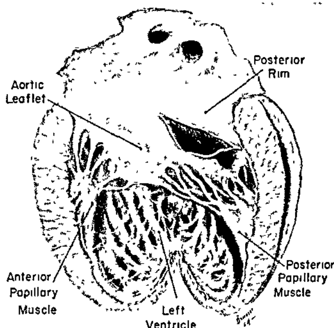


Fig 1. Detachment of posterior cusp of mitral valve.

process No emboli were detected in any of the animals. Figure 1 shows this procedure.

SUTURE OF ATRIAL PEDICLE TO PAPILLARY MUSCLE

The early conversion of intracardiac tissue slings and pericardial pedicle flaps to vascular fibrous cords is well known.[1,3] In spite of this, some clinical success has been reported with pericardial pedicle flaps.[4] The chances for anything but the temporary relief of mitral insufficiency so treated must

Table 2. Suture of Atrial Flap to Papillary Muscle

NO	LENGTH OF SURVIVAL	CAUSE OF DEATH	REMARKS
1	1 month	Sacrificed	Atrophy of flaps
2	6 weeks	Sacrificed	Mod. atrophy of flaps
3	6 weeks	Sacrificed	Minimal thrombus Atrophy of flaps
4	2 months	Sacrificed	Atrophy of flaps
5	1 month	Sacrificed	Atrophy of flaps
6	4 days	Hemothorax	No atrophy or thrombus
7	Op death	Recurrent ventricular fibrillation	————
8	2 months	Sacrificed	Atrophy of flaps
9	2 months	Sacrificed	Atrophy of flaps
10	Still alive	————	————
11	Still alive	————	————
12	3 weeks	Sacrificed	Mod atrophy of flaps

be limited. Prosthetic baffles appear to work better than the various autogenous flutter valves.[5,6]

Suturing the left auricular appendage either completely or as a pedicle against the ventricular wall undoubtedly developed from closed invagination techniques for interatrial septal defects.[7,8] Early relief of mitral insufficiency by the method of complete invagination has been reported clinically; however, late results were discouraging.[9] Shrinkage of pedunculated atrial flaps has been noted experimentally, but the degree of shrinkage was not considered to be as hopeless as that of other autogenous slings and flaps.[10]

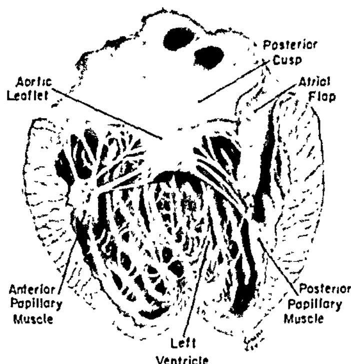


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3. Pedicle flaps of left auricle and atrium sutured to either papillary muscle through the mitral valve undergo late shrinkage and appear ineffective.

4. The use of a papillary muscle to anchor prosthetic material hinged at

the mitral ring is indicated by these experiments.

5 Detachment of the posterior rim of the mitral valve, though temporarily effective for mobilization, is later nullified by healing processes.

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USE OF THE RIGHT AURICLE AS A PUMP FOR THE PULMONARY CIRCUIT*

HERBERT E. WARDEN, RICHARD A DE WALL, AND RICHARD L. VARCO

The current progress in the management of congenital heart disease is not sustained in the case of the patient with congenital tricuspid disease. Their prognosis still remains poor Most present-day surgical techniques for the treatment of this disease seek to increase the blood flow through the pulmonary circuit via a systemic shunt type procedure This results in an inefficient recirculation to achieve an ample volume of oxygenated blood for the body The preferable goal would seem to be application of a direct pumping mechanism to force venous blood through the pulmonary circuit

the pumping ability

of e pulmonary artery.

pumping potentials

of the right auricle.† At first this was done by making an end to side, right

* From the Department of Surgery, University of Minnesota Medical School, Minneapolis This study was supported by funds from the Minnesota Heart Association, Graduate School of the University of Minnesota Medical School, and University of Minnesota Department of Surgery Cardiovascular Research Fund.

† When the present study was well under way the work of Dr Radbard was called to our attention. He and his associates were also impressed by the possibilities of venous perfusion of the pulmonary bed Rodbard, S, and Wagner, D: Proc. Soc. Exp Biol & Med, 71 69-71, 1949.

auricular appendage–pulmonary artery anastomosis followed by obliteration of the right ventricular cavity by means of multiple sutures placed through the right ventricle catching the ventricular septum and then brought back through the ventricular wall. As these were tied the walls of the right ventricle became approximated and the size of the ventricular cavity diminished. This method was discarded since it produced an inconsistent degree of outflow track obstruction. In the next series, the anastomoses were constructed as above and the tricuspid valve sutured from within under direct vision, either by means of venous inflow occlusion[1] or with the aid of a donor and controlled cross circulation.[2] The two procedures were done in a single stage and, although there were survivors, the mortality was pro-

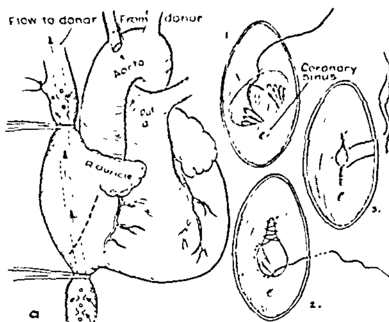


Fig. a. Shows the catheters in place, the vena caval occluding tapes and the site of the auriculotomy. The vena cava catheter carries venous blood to the pump and thence to the donor while the catheter in the innominate artery supplies arterial blood from the donor and the extracorporeal circuit. *1* shows the beginning tricuspid suture at the medial angle and running laterally (2), and the completed tricuspid stenosis with the purse string brought out through the right ventricle (3).

hibitive. Because we felt, too, that it would be desirable to produce one of the pathologic features of congenital tricuspid stenosis, namely right auricular hypertrophy, before performing the corrective procedure, the following method has been more thoroughly tested

METHOD

First Stage—Tricuspid Stenosis (Fig. a). Healthy mongrel dogs weighing from 10 to 25 kilograms were anesthetized with 2½ per cent Pentothal Sodium intravenously after having been premedicated with morphine and atropine. All animals had endotracheal intubation and were artificially respired during the operative procedure. The details concerning the controlled

...y have been de-

...was made in the

...interspace and the right auricle of the study animal opened (Fig. a).

The medial angle of the tricuspid valve was grasped with an Allis forceps, and a 3-0 braided silk suture then placed at this medial angle and sewn laterally until the mid-portion of the valve was reached (Fig. a1 and a2). Here it was tied. A similar suture was then started at the lateral angle of the valve adjacent to the coronary sinus orifice and run medially toward the previously laid stitches, ending with a stenosed orifice in the center of the tricuspid valve of about 8 to 14 mm. diameter This was measured with a Hegar dilator. When the orifice was stenosed to the desired size the lateral suture was tied The free ends of the suture were used to purse-string the remaining orifice and then brought out through the right ventricle just below the coronary sulcus, taking care to avoid the right coronary artery (Fig a3). These free ends were tied loosely with a large loop so that there was no constriction of the remaining tricuspid orifice. These ends remained free in the pericardium. After the auriculotomy had been closed with a continuous 3-0 suture, the venae cavae were released and the pump stopped. The chest was closed in layers, with penicillin and streptomycin in the chest cavity. Each animal was injected intramuscularly daily with penicillin and streptomycin for seven days, and intravenously with penicillin for five days.

Second Stage—Right Auricular-Pulmonary Artery Anastomosis (Figs. b,c,d,e) Based on the animal's postoperative condition as well as the intermediate venous pressure values following the creation of tricuspid stenosis, the second operative stage was delayed from two weeks to two and one-half months. At this stage the dog was prepared and anesthetized in the fashion as before. Through a left thoracotomy in the fourth interspace the pericardium was incised widely anterior to the left phrenic nerve. Within this sac the tricuspid purse string was located The right auricular appendage at this stage was usually enlarged about 2 to 2½ times normal size. The fat pad and adventitia intervening between the aorta and pulmonary artery were excised so that the anterior surface of the ascending aorta and the anteromedial surface of the pulmonary artery up as far as the take-off of the right main pulmonary artery were clear of any superfluous tissue. The tip of the right auricular appendage was grasped, stretched with a lung forceps, cross clamped with a ductus clamp (Fig b), and anastomosed to the medial anterior surface of the main pulmonary artery just above the pulmonary valves where it had been isolated by means of a spoon Glover clamp placed on the long axis of this vessel The occluded portion of the pulmonary artery was incised with a No. 11 Bard Parker knife for a distance of approximately 2 cm. (Fig c). A slightly smaller incision was then made along the distal margin of the right auricular appendage (Fig. b) The anastomosis of these structures was effected using a 6-0 double armed arterial silk suture employing a continuous over and over stitch (Fig. d). After opening the anastomosis it was usually apparent that there was flow through the new union from the pulmonary artery into the right auricle, as evidenced by a thrill over the auricular appendage. The final step (Fig. e) consisted of complete occlusion of the tricuspid valve by tying down the previously placed purse-string suture that had been led into the pericardial sac during the first operation After completion of this maneuver a thrill was palpable over the pulmonary artery and the direction of the shunt flow was reversed Also, the right ventricle now became much softer. The pericardium was approximated loosely, penicillin and streptomycin were placed in the chest cavity, and this was closed as indicated earlier.

The venous pressures listed (Table 1) represent, we believe, largely right auricular pressures as recorded on a Sanborn Poly-Viso Recorder via a No. 8 ureteral catheter inserted into either jugular vein and passed down into the right auricle. These values were determined as often as before and, after making the tricuspid stenosis, again once and usually twice between operative stages, then before and after construction of the right auricular anasto-

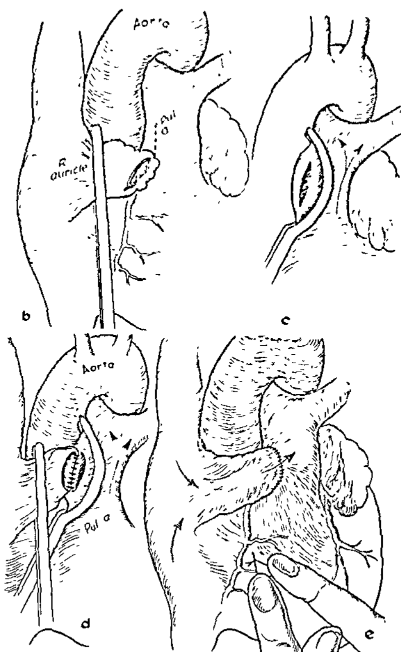


Fig. b. The right auricular appendage is cross clamped and the tip incised.
Fig. c. The anteromedial surface of the main pulmonary artery is occluded and incised preparatory to anastomosis to the right auricular appendage.
Fig. d. The right auricular appendage and the main pulmonary artery are approximated and the posterior margin of the anastomosis has been completed.
Fig. e. The right auricular appendage–pulmonary artery anastomosis is complete and the purse-string about the stenosed tricuspid orifice is being tied.

mosis, next at the completion of the second stage; and finally as frequently in the follow-up course as seemed indicated by the pressures noted.

RESULTS

Thirty-two dogs have been operated on to date in this study. Ten were lost early through technical errors in the developmental phase of the study. Four additional animals had interatrial defects made at the same time the tricuspid stenosis was made In three of these the septal defects were too large and/or the tricuspid stenosis too great and they died within 24 hours. The fourth animal survived 14 days, dying after the tricuspid purse string was tied at the second operation, and at autopsy had an old thrombus beginning at the site of the septal defect and filling the entire right auricular

Table 1. Venous Pressures in Dogs with Tricuspid Stenosis and Right Auricle–Pulmonary Artery Shunts*

DOG NO	INITIAL	BEFORE 2ND STAGE	FOLLOW-UP	COMMENT
141	8	27	14	Alive and well, 4 months
170	5	25	11	Died 25th day after 2nd stage. Granuloma obstructing inferior vena cava
349	6	25	13	Died 27th day—tricuspid purse-string broke Died right heart failure from reversal of right auricle–pulmonary artery shunt
359	5	35		Died 40th day after 1st stage during induction preparatory for 2nd stage Tricuspid measured 9 mm
1088†	6		18	Died 8th postoperative day. Anasarca shunt only 7-8 mm in size
1168†	5		10	Killed in dog fight on 30th postoperative day Gross pneumothorax—tricuspid 90% closed
1244†	3		10	Alive and well, 1 year
1270	6	28	15	Alive and well, 3 months

* All pressures are in terms of cm sodium citrate

† These animals were operated upon early in the study before we began recording pressures taken between stages

appendage and the superior vena caval system. Three other dogs died within 12 hours after the first stage operation of too complete a tricuspid stenosis Five animals are living and well, awaiting the second operation.

The remaining eight animals are the primary concern of this report. Their venous pressure alterations are recorded with a brief comment in Table 1 All of these animals had both operations completed with the exception of dog No. 359. This animal succumbed during induction of anesthesia preparatory to performance of the right auricle–pulmonary artery shunt and has been included to indicate the degree of increase in venous pressure following the first operation carried out 40 days before In spite of the comparatively high venous pressure present, the animal was quite active, maintained a stable weight and exhibited no fluid accumulations. This canine's tricuspid orifice measured 8 to 9 mm. at autopsy.

As noted in Table 1 the remaining seven animals had right auricular pressures measured before production of the tricuspid stenosis and after the

auricle–pulmonary artery anastomosis was made and the tricuspid purse-string suture tied. Four of these animals had pressure measurements made between the first and second procedures.* In each of these dogs there was a significant elevation in venous pressure following the first operation, with 30 cm. of citrate being the greatest rise and 19 cm. of citrate the least; the average rise was about 22 cm. of citrate. In the four dogs subjected to both procedures who had between-stage measurements made, there was an average decline of 13 cm. in pressure following production of the auricle–pulmonary artery shunt. All animals had venous pressure values, after completion of the second procedure, that were higher than their preoperative level with the average pressure 7.4 cm. higher than the initial recording.

One dog, No. 1088, had a course typical of severe clinical tricuspid stenosis with a rapid weight gain, ascites, hydrothorax and death on the eighth postoperative day. He also had the highest postoperative venous pressure. At autopsy the pulmonary artery side of his shunt measured but 7 mm. in diameter and we believe that this probably accounted for his steady deterioration.

After production of the right auricular shunt into the pulmonary artery we found that it was important to close off the tricuspid valve to avoid a reverse flow through the shunt from pulmonary artery to right auricle. Under the above conditions an anemia of the pulmonary bed develops with overloading of the right side, heart failure, and death. This occurred in dog No 349.

DISCUSSION

This report covers our initial experimental efforts with the problem of tricuspid atresia. We have attempted to produce experimentally only four of the common pathologic features of this disease, i.e., tricuspid obstruction, right auricular hypertrophy and dilatation, elevated vena caval pressure and diminished pulmonary blood flow. Suture of the tricuspid orifice in the dog under direct vision utilizing either the technique of inflow occlusion or controlled cross circulation to gain access into the heart permits the production of a high grade tricuspid stenosis At a later stage when the animal has partially adapted to these pathologic changes we have attempted to modify favorably his cardiac disabilities by creating a shunt between the right auricle and the side of the pulmonary artery. Under the latter conditions the venous return to the heart now completely by-passes the right ventricle en route to the pulmonary artery.

The results of the study indicate that the production of a shunt between the right auricle and the pulmonary artery consistently results in a reduction of the resting venous pressure hitherto elevated by tricuspid obstruction. Additional quantitative flow studies are required to provide more accurate volumetric dimensions to the clearly recognizable tolerance of these dogs to tricuspid stenosis or atresia after an auricular pulmonary artery shunt has been completed.

In conclusion, it is our opinion that although a right auricle–pulmonary artery shunt shows promise for the future treatment of congenital tricuspid disease it must be subjected to further extensive laboratory evaluation.

* Dogs 1088, 1168, and 1244 were done early in the series before we had adopted routine pressure measurements between the first and second stage operations.

SUMMARY

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under
direct vision using either the technique of inflow occlusion or controlled cross circulation

2 A partial regression in the venous pressure, elevated by virtue of a tricuspid stenosis, results after producing a shunt from the right auricle directly to the pulmonary artery.

3. The possibility of future application of this method to the treatment of tricuspid atresia we feel must await more experimental investigation.

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EXPERIMENTAL CLOSURE OF INTERVENTRICULAR SEPTAL DEFECTS AND FURTHER PHYSIOLOGIC STUDIES ON CONTROLLED CROSS CIRCULATION*

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Controlled cross circulation has been employed in this clinic over the past nine months for the direct vision intracardiac correction of congenital heart defects in twenty-one patients † Concomitantly, work has continued in the laboratory to further evaluate this method of performing prolonged open intracardiac surgery. Many of the problems that were evident in the early experimental work, such as fibrillation, have not been encountered in the clinical experience to date, while the latter has emphasized certain features which had not been explored. Among these was the increase in the donor's respiratory minute volume necessary to maintain his alveolar pCO_2 within

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† Since the presentation of this paper an additional 11 patients have been operated upon, making the present total 32 Of these, 22 were suture closure of ventricular septal defects, with 7 deaths, 6 were for the curative treatment of the tetralogy of Fallot defect, with 3 deaths, 2 were for correction of atrioventricularis communis defects, with 1 death; and 1 patient with a complicated defect (pulmonic stenosis, interatrial septal defect, and anomalous pulmonary drainage) did not survive corrective surgery. There has been no donor mortality in these 32 operations.

normal limits as monitored by a portable mass gas spectrometer during the period of perfusion.[1] This increased respiratory demand and its relationship to post-perfusion alterations in acid-base relationships was implied by previous work[2] but at that time had not been investigated.

Therefore, experimental efforts have been mainly directed at further [illegible] in both donor and recipient [illegible] work has continued to more [illegible] to support the heart during the trauma of intracardiac surgery.

METHOD

Mongrel dogs weighing from 9 to 23 kilograms were used as recipients, while the donors ranged from 14.8 to 36.6 kilograms in weight. Each animal received 4.5 mg. morphine per kilogram and 1/100 grain of atropine preoperatively. Pentothal Sodium (2½ per cent) intravenously was used as an anesthetic agent. Each animal had an endotracheal tube inserted and the recipient was respired by a mechanical respirator during the period his chest was open. The endotracheal tube of the donor was attached to a special Y adaptor which, in addition to connecting the endotracheal tube with a mechanical respirator,[3] also had an outlet to accommodate the sampling tube to the mass gas spectrometer for continuous sampling of respiratory gas. Also inserted in the respiratory circuit was a Bennett Ventilation Meter.* Under these conditions the respiratory rate and volume could be controlled and at the same time correlated with the partial pressure of the carbon dioxide in the alveolar air.

The remainder of the technical aspects of the method were identical in every respect to that used in the earlier studies and described in detail elsewhere.[2]

PHYSIOLOGIC RESULTS

There were twenty-four dogs used in this group (three animals served once as recipient and once as donor while another was a donor twice), representing fourteen study operations. In each the recipient was subjected to a right thoracotomy and thirty minutes of controlled cross circulation during which time the entire inflow to the right heart was completely occluded. No cardiotomies were performed in these animals. Before, during, and after the period of perfusion, blood specimens were drawn for venous and arterial oxygen and the carbon dioxide content, arterial *p*H, hemoglobin, hematocrit, and plasma hemoglobin determinations. However, for the sake of brevity and because the results of these determinations were similar to those of previous reports,[2, 4] all except the alterations that occurred in the arterial *p*H have been omitted from this discussion. Control arterial *p*H samples were drawn from both the recipient and the donor animals just before the pump was turned on; additional specimens were drawn at 10 minute intervals during the actual perfusion. These were drawn from the extracorporeal arterial circuit and, because of the continuity of their circulations, were interpreted as representing the *p*H of each animal.

In addition, each donor's respiratory minute volume and alveolar pCO_2 was monitored continuously and in many experiments the aortic and vena

* Manufactured by the Monaghan Co., Denver, Colorado.

Table 1. Relationship of Recipient and Donor Body Weight and Perfusion Rate to Variations in Donor's Respiratory Minute Volume (R.M V.) and the Resultant Changes in His Alveolar pCO_2 and Blood pH during Thirty Minutes Controlled Cross Circulation

					Before by-pass				During by-pass								
									10 min			20 min.			30 min.		
Study	Recipient No Wt.	Donor No Wt.	Pump flow cc/min.	Flow* cc/kg/min	Recipient pH	R.M V †	pCO_2†	Donor pH	R M V †	pCO_2†	pH‡	R M.V †	pCO_2†	pH‡	R M V †	pCO_2†	pH‡
A	#406 9 kg	#1063 22 kg	380	42	7.675	4.25	40	7 354	4.5	43 5	7 295	19 0	21 8	7 502	19 0	18 2	7.514
B	#345 11 kg.	# 882 23 kg.	420	38	7 527	8 5	42 8	7 320	8.5	55 5	7.208	16.4	25 8	7 406	20 4	25 4	7.421
C	#866 17 8 kg.	# 864 16 1 kg	640	36	7.507	5 5	32 7	7.363	8 0	29 0	7 332	15.8	18.4	7.150	7 3	29 0	7.317

* These figures refer to flow in cc/kg. recipient weight/minute.

† Donor's respiratory minute volume in liters/minute and the pCO_2 in mm. Hg in his alveolar air During the period of cardiac by-pass the recipient was not ventilated.

‡ Samples drawn from the arterial pump circuit at the times indicated.

caval pressures of both donor and recipient were recorded simultaneously throughout the perfusion.

Arterial pH Changes. The respiratory demand placed on the donor by the size and the perfusion rate of the recipient was reflected by the changes in the arterial pH and the donor's respiratory minute volume and alveolar pCO_2. The data from three representative study runs are tabulated in Table 1. The donor in study A was almost 2½ times as heavy as the recipient. In this instance maintenance of his R.M.V. at essentially the control level during the first 10 minutes of the run netted only a slight increase in alveolar pCO_2 but a pH of 7.295. Throughout the remaining 20 minutes of the study he was hyperventilated (R.M.V. 19), which resulted in a progressive fall in the pCO_2 and an overcompensating rise in the blood pH to 7.514. In contrast to these changes are those which occurred in study B, where the weight differential was only a little over two times in favor of the donor and the perfusion rate per kilogram per minute was less. *Here when the*

Table 2. Simultaneous Donor and Recipient Aortic and Vena Caval Pressures during Cross Circulation*

	BEFORE BY-PASS	CARDIAC BY-PASS		AFTER BY-PASS	
	CONTROL	8 MIN	28 MIN.	30 SEC	3 MIN.
Donor No. 1949†					
Ht. Rate	96	65	78	118	90
Pressures					
Aorta	175/125	210/130	215/175	185/140	197/160
I V C.	9	12	15	7	6
Recipient No. 1921†					
Ht. Rate	208	180	162	100	162
Pressures					
Aorta	138/119	96/89	71/69	112/89	111/99
I V C.	15	28	31	15	16

* Aortic pressures refer to mm Hg and I.V.C. to cm H_2O.

† Donor wt. 24 kg. and recipient wt. 12.3 kg. Perfusion at 375 cc./min or 30 cc./kg recipient wt./min.

R M.V. during the first third of the cardiac by-pass period was unchanged from the control volume there resulted a marked rise in pCO_2 to 55.5 and a pH of 7.208. However, by increasing the R.M.V. almost twofold the pCO_2 reversed and the pH became more alkaline until, after 20 minutes of perfusion, the pH was 7.406. Finally, during the last 10 minute period the R.M.V. was regulated to maintain the pCO_2 at its 20 minute value and the pH approached a more normal value of 7.421. Study C represents the situation where the donor was slightly smaller than the recipient. It required rather marked hyperventilation (2.8× control R.M.V.) of this relatively small donor to produce a satisfactory elevation of pH (7.450 at 20 minutes with a pCO_2 of 18.4), and this rapidly reversed when the R.M V. was allowed to fall off.

Blood Pressure. Table 2 summarizes the data obtained from the continuous simultaneous measurements of aortic and vena caval pressures in both the donor and the recipient animals during controlled cross circulation measured by a catheter–strain gauge system and recorded on a Sanborn Poly-Viso Recorder. The aortic pressure of the donor showed a definite rise

at the onset of the perfusion. This elevation persisted throughout the run and reached maximum at approximately 28 minutes. The donor's vena caval pressure paralleled that of the aorta and both promptly returned to pre-perfusion levels when the pump was stopped. The alterations in cardiac rate showed an inverse relation to the pressure changes. Concomitantly, the recipient's cardiac rate was relatively stable while his vena caval pressure rose to 34 cm. H_2O and his aortic pressure fell commensurate with the perfusion rate as alluded to in the previous work.[2, 5] Again it was striking how promptly the recipient's measurements returned to their control level after 30 minutes of total cardiac by-pass.

OPERATIVE RESULTS

In this group 92 dogs were used: 50 as recipients and 42 as donors, with 8 animals serving as donors twice. Right ventriculotomies with creation and closure of high interventricular septal defects were performed in 44 of the recipients while the remaining 6 had right auriculotomies and interatrial septal defects made and closed under direct vision. Out of this entire group 32, or 64 per cent, survived.

Table 3. Experimental Intracardiac Operations by Controlled Cross Circulation*

BLOOD FLOW CC /KG BODY WT /MIN	NO DOGS	SURVIVORS
30 to 47 cc	10	7 (70%)
20 to 30 cc	15	10 (66⅔%)
10 to 20 cc	25	15 (60%)
	50	32 (64%) (Over-all)

* 44 I.V S D, 6 I A S D

The perfusion rates employed in this operative group ranged from 10 to 47 cc per minute per kilogram body weight of the recipient. These flows have been correlated with the survival rate in Table 3 In 10 dogs the rate of blood flow ranged from 30 to 47 cc per kilogram per minute. Seven of these (70 per cent) survived Of the remaining 45 animals, 15 received 20 to 30 cc of blood per kilogram per minute with 10 (66⅔ per cent) survivors, while in 25 perfusion rates ranging from 10 to 30 cc. per kilogram per minute were used during the period of cardiac by-pass and 15 (60 per cent) lived. The 6 animals with interatrial defects were equally divided among the three groups.

Ventricular fibrillation occurred 28 times (56 per cent) in this operative series. The onset was usually during the period the intracardiac procedure was in progress and no attempt was made to defibrillate or massage until it was completed and the cardiotomy was closed The incidence of fibrillation and the salvage rate are correlated with the perfusion rates in Table 4. Of the 10 dogs perfused with 30 to 47 cc. of blood per kilogram per minute, 6 (60 per cent) fibrillated and all 6 (100 per cent) were successfully defibrillated. In the intermediate group (20 to 30 cc./kg /min), 9 (60 per cent) went into ventricular fibrillation of which 6 (66⅔ per cent) were converted to a normal rhythm and survived, while in the last group of 25

dogs that received the least flow (10 to 20 cc./kg./min.), 13 (52 per cent) developed the arrhythmia and only 6 (46 per cent) could be salvaged.

DISCUSSION

The present efforts represent a continuing approach to some of the problems suggested by earlier studies. The development of an acidosis during cardiac by-pass appeared in the autogenous lobe series[4, 5] and also occurred in the previous cross circulation studies. In addition, this earlier work revealed that the acidosis resulted predominantly from an accumulation of fixed acid metabolites with only a slight rise in the arterial CO_2 tension but a marked increase in the lactic acid content.[4] From the data herein presented it is evident that this metabolic depression of the pH develops in the presence and in spite of an alveolar pCO_2 tension that is kept within normal limits by regulation of the donor's respiratory minute volume. However, these results also indicate that it is possible to compensate for this metabolic acidosis and elevate the pH toward (and at times exceed) normal values by increasing the donor's R.M.V. to the extent that the alveolar pCO_2 is below the normal range. These observations have also been suggested

Table 4. Incidence of Fibrillation in Relation to Perfusion Rates

BLOOD FLOW CC./KG. BODY WT./MIN.	FIBRILLATION	SURVIVORS
30 to 47 cc. (10 dogs)	6 (60%)	6 (100%)
20 to 30 cc (15 dogs)	9 (60%)	6 (66⅔%)
10 to 20 cc (25 dogs)	13 (52%)	6 (46%)
Total 50 dogs	28 (56%)	18 (64%)

by our clinical experience with cross circulation. Under these conditions the donor's respiratory minute volume has generally been increased 2 to 3 times its control figure in order to maintain the CO_2 tension within normal limits during the time the patient has been dependent upon him for oxygenation.[6] It is of interest to note, however, that in the human being this degree of R.M.V. increase has been relatively consistent regardless of the weight relationship of the patient to donor.

The alterations in arterial and venous pressures reported are another feature that has been observed at the clinical level. The number of direct intravascular determinations that have been made on human patients has, by necessity, been few and they have not been as complete as those done in the laboratory However, those that have been collected attest the experimental findings.

The results of the operative series of animals reconfirm the observation that the higher rates of perfusion afford the heart greater protection against, or reserve to withstand, the trauma of cardiotomy and intracardiac operations as indicated by the better survival rates in these groups. This is particularly true of operations within the ventricles. It has been conspicuous in the laboratory that auriculotomies and interauricular procedures can be

...quired for entrance

... there is a definite

advantage demonstrated by the higher flow rates with respect to salvage of animals that develop ventricular fibrillation (the rate of successful defibrillation being directly proportional to the perfusion rate). In contrast to the human heart, the canine heart is extremely irritable and prone to fibrillate at the slightest provocation. The incidence of this arrhythmia is essentially the same for all three flow groups in this study with the possible exception of the 10 to 20 cc. group in which it occurred 52 per cent of the time However, we feel that this may well be more apparent than real, for this group is unduly weighted with animals operated on early in the study when, owing to technical errors, the dogs often succumbed from causes other than fibrillation.

SUMMARY

1. A method for performing intracardiac operations under direct vision in a dry field by means of controlled cross circulation is presented. By means of a mechanical pump, blood is transported from the arterial system of a donor individual, who serves as an oxygenator, to the arterial system of the recipient. At the same time, a like amount of blood from the latter's venous system is transferred to the venous circuit of the donor.

2. During the period of total cardiac by-pass by this method there develops a metabolic acidosis [illegible] of, a normal alveolar pCO_2 o [illegible] his respiratory minute volume. [illegible] by increasing the donor's respiratory minute volume to the point where his alveolar pCO_2 is below the normal range.

3. The aortic and vena caval pressures in the donor rise during the period of perfusion, reaching a peak at approximately 28 minutes. The vena caval pressure of the recipient also goes up under these conditions but his aortic pressure falls during cardiac by-pass. All pressures rapidly return to preperfusion levels after the pump is stopped.

4 A series of 50 dogs were subjected to intracardiac operations (44 I.V.S D., 6 I A S D) with an over-all survival rate of 64 per cent. The higher perfusion rates enable the heart to withstand surgical trauma better than the lower ones and consequently net a higher percentage of survivors In addition, the salvage rate of animals that fibrillate is directly proportional to the rates of perfusion.

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A PARABIOTIC BLOOD PUMP*

LESTER BLUM, SAMUEL J. MEGIBOW, AND WILLIAM M. NELSON

Intracardiac surgery ideally requires a bloodless field. Complete exclusion of the heart from the general circulation, without interruption of coronary flow, may be achieved in one of two ways: either a pump-oxygenator system or a form of parabiosis in which a companion animal assumes the circulatory burden.[1] This report describes a satisfactory apparatus for employment of the latter technique.

APPARATUS

Our device is essentially a dual-acting pump so constructed as to permit volume equilibration of two independent channels of blood flow. The propelling action is accomplished by the alternate expansion and contraction of two flexible, non-wetting, silastic bladders. These are ellipsoidal in shape, tapering integrally into tubing of the same material. One bladder serves the arterial system, the other the venous.

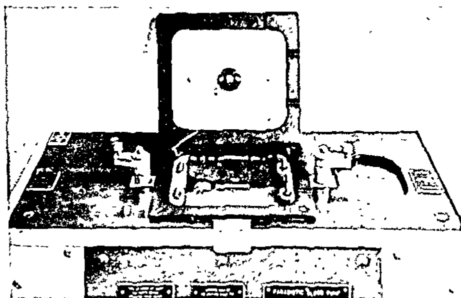


Fig 1 View of bladder platforms with chamber cover lifted.

The two bladders rest on platforms in a rectangular metal chamber with an airtight, transparent plastic cover (Fig. 1). In operation, the chamber is evacuated up to 120 mm. Hg, which serves to help expansion as the platforms descend during the intake portion of the stroke. The output or systole is obtained by ascension of the platforms which compress the bladders against the chamber cover. The bladders are never completely emptied during this efflux portion of the cycle so that the retained blood reserve serves as a buffering mechanism to minimize turbulence and blood cell destruction.

* From the laboratories of the Mount Sinai Hospital, New York. This study was aided by a grant from the Shal-Aide Society

Valving is effected by rods or stoppets lying within the vacuum chamber where the bladders join the tubing. These stoppets alternately pinch each bladder neck against the cover. They are individually spring-loaded to provide sufficient pressure for cutoff without damaging the silastic material. By their external valving action, they preclude fluid backlash.

The platforms on which the bladders rest are connected to the pumping mechanism by vertical plungers. These are operated by two parallel con-

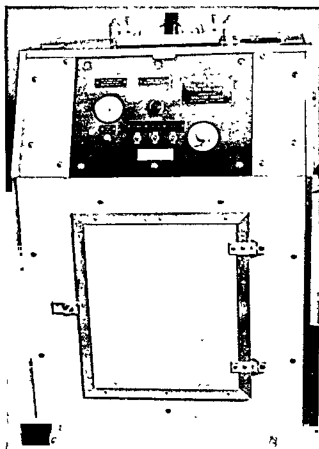


Fig 2 Front view of apparatus.

necting rods driven from a common crankshaft Three mechanical adjustments are incorporated into the unit·

1. The main stroke adjustment moves the crankshaft in a direction parallel to the rods, thus altering their effective length or the stroke distance of both plungers. This controls total flow

2 and 3. The arterial and venous stroke adjustments can be separately made by moving the fixed or fulcrum end of either connecting rod to alter the lever-arm ratio, hence the stroke, of either platform. This latter adjustment is individual and makes possible independent alteration of flow in either arterial or venous circuit during delivery.

The pump is driven by an electronically controlled variable speed motor. This consists essentially of a shunt motor operated through a power unit

in which a selenium rectifier supplies the field current at constant voltage. The armature current is furnished by a type C-3J thyratron and is varied by changing the grid bias. This latter control is a standard 1 megohm potentiometer. This results in an adjustable pulsatile flow through both blood channels.

The vacuum system depends on a "Pressovac Model No. 4" vacuum pump driven by a ¼ hp. A. C. motor. The vacuum pump feeds from a storage tank of one and a half gallons capacity to the bladder chamber. A spring-loaded relief valve connected to the bladder chamber is included and has an adjustable screw for maintaining the desired degree of vacuum in the chamber.

The speed of the pump motor is not uniform, but is controlled by a cam-operated switch on the crankshaft so that on the intake portion of the stroke the speed is retarded, permitting additional time for the bladders to fill with minimal turbulence. The ratio of intake to output time is not constant



Fig. 3. Bladder, tubing and catheters.

but varies with the pulse rate, its highest value obtaining at the maximal rate.

The entire machine is housed in a rectangular cabinet (Fig. 2) which is mobile. With the exception of the stroke adjustment knobs on the side, all controls are grouped on a front instrument panel.

Each channel, arterial and venous, thus consists of bladder, tubing and catheters at either end (Fig 3). The silastic bladder and tubing can be autoclaved repeatedly. The polyethylene catheters must be sterilized chemically. Bladder-tubing-catheter joints are so constructed as to avoid overhang and sudden change in caliber.

Blood destruction as evidenced by hemolysis appears minimal (Table 1). We originally used 2 mg. of heparin per kilogram. Occasionally we dispensed with its use, the only heparin employed being a very dilute instillation in the catheters during their insertion.

The maximal flow is limited by the caliber of the catheters. Each bladder

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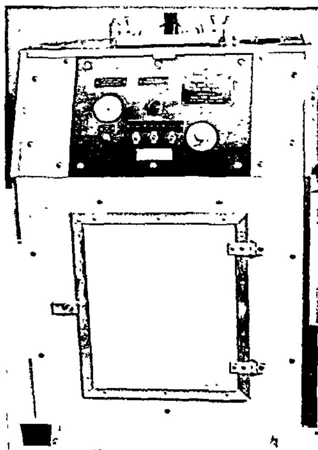


Fig. 2 Front view of apparatus.

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with the right atrium. The blood is conducted from the catheter through the silastic tubing leading to the venous bladder and is pumped out again through the other arm of silastic tubing to the venous catheter of the donor which lies in either the femoral or jugular vein.

The parallel arterial circuit leads from catheters in the femoral arteries of the donor dog through its own silastic tubing and bladder to catheters in the femoral and carotid arteries of the subject dog. Thus, when the heart of the subject dog is excluded, all the venous blood returning through the venae cavae is diverted to the donor dog for oxygenation while an equal amount of arterial blood is being simultaneously pumped from the donor dog back into the arteries of the subject dog.

We have succeeded in completely excluding the dog heart for periods up to ninety minutes without fibrillation. The aortic leaflets are locked by the pulsatile nature of the flow which simulates the natural state.

A CONSIDERATION OF THE DYNAMICS OF BLOOD FLOW IN THIS SYSTEM

It is customary to attempt to simplify physical expressions of blood flow by considering blood as a Newtonian fluid. Poiseuille's equation is not applicable since it considers blood as an elastic body of unit constants. We feel that blood differs from an elastic solid in that it does yield to a shearing stress. Furthermore, this equation is based on a concept of laminar flow wherein the viscosity of each layer is integrated during its slow, steady passage through a small bore channel.[2] Any departure such as pulsation or local eddies or change in lumen invalidates its application.[3]

Since it is not convenient to relate the displacement of the blood with the stress tensor even if the displacement were kept constant, the shearing stress of the fluid would vary with time. It is simpler to express the behavior of blood flow in terms of velocities rather than displacements. Two approaches may be used: one which gives the velocity of each blood particle at each instant of time, and another which gives the fluid velocity at each point in space at each instant of time. The two types of motion resemble each other from the viewpoint of a non-Newtonian fluid and the continuum picture. Some of the forces are internal owing to other particles nearby, these determine compressibility. Other forces are external, acting more or less equally on all particles in a given region. This concept attempts to embrace the phenomena of hemolysis, coagulation and unexpected variations in flow which concern the surgeon interested in the extracorporeal circulation of blood.

In plotting the flow lines of the average paths of blood particles,

$$\Delta x/vx = \Delta y/vy = \Delta z/vz \quad . \; . \; . \; . \; . \; . \; . \; . \; . \; . \; .$$

The number of path lines intersecting a given surface which is equal to the outflow integral $\int v \cdot \Delta a$ across the surface is also equal to the average flow across the surface. If no eddying exists, the flow lines are perpendicular to the equipotential surfaces and constitute a natural coordinate system.[4, 5]

Of the many flow factors to be considered, one is concerned with the relation between net outflow of mass from volume elements Δx, Δy, Δz . . . . The latter is equal to the loss of mass $\rho \Delta x \Delta y \Delta z$. For instance, the acceleration of blood constituents x, y, z at time t is:

with attached tubing without catheters is capable of two liters per minute Calibration to equalize arterial and venous channel delivery must be done with the selected catheters attached.

Because of the disproportion between cross-section of the main venous

Table 1. Hemolysis

DOG	MG HBC PER 100 CC OF PLASMA AT DELIVERY RATE OF 800 CC PER MIN.						
NO	PRE-OP	20 MIN	40 MIN	60 MIN	80 MIN	100 MIN.	140 MIN.
487	39	15	19	16	31		
439	2 5	6	9	10	11		
553	240	276	232	246	210	187	180
531	44	53	54	54	50	63	
563	37	26	27	25	30		
488	104	100	64	82	84	108	88
571	34	54	45	42	48		

channels and the smaller arteries of the neck and extremities, two catheters have been used at each end of the arterial channel.

METHOD.

The animals, identified as subject and donor, are placed side by side with sufficient interval (about three feet) for the apparatus (Fig 4). A catheter

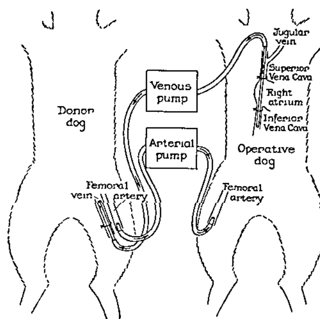


Fig 4 Schema of blood flow and arrangement of animals in parabiotic technique (By permission of Journal Mt. Sinai Hospital)

is inserted in the jugular vein of the subject dog and passed down the superior vena cava until its tip lies in the inferior vena cava. Its apertures are so placed that the entire venous return to the heart can be diverted into the catheter by ligatures passed about the venae cavae at their junctions

A NEW METHOD FOR THE DRAINAGE OF BLOOD FROM THE OPEN HEART DURING TOTAL BY-PASS OF THE HEART AND LUNGS*

JAMES A. HELMSWORTH, LELAND C. CLARK, JR., SAMUEL KAPLAN, AND GLYNN FORD**

The problem of draining away the coronary venous blood which may flood the opened cardiac chambers confronts all experimentalists who carry out intracardiac procedures under direct vision. The authors have channeled this coronary venous drainage back into the extracorporeal apparatus in all of their experiments. There can be no doubt, however, that the reinfusion of this venous blood may be hazardous, and errors in handling this problem have caused the death of the animal in many perfusions in this laboratory. It is true that this report deals with the problem as it is encountered in a very specialized laboratory project in which the coronary venous blood is passed through a heart-lung apparatus before it is pumped back into the animal's circulation. Nevertheless, certain information gained may apply to any method for cardiotomy in which the collected coronary venous blood is reinfused into the animal's arteries.

An earlier report from this laboratory gave measurements of the volume of coronary flow from the opened heart during total by-pass of the lungs and the cardiac chambers.[1] It has not been exceptional to find that the coronary flow comprised 15 per cent of the total perfusion flow per minute. (In all of the experiments there has been a tendency to maintain a relatively high minute perfusion flow, 75 to 100 cc per kilogram of body weight, in an effort to duplicate certain measurements of cardiac output in dogs.)[2] Thus, in a dog of average size (15 kg.) the coronary flow in the conditions of these experiments is in the range of 150 to 200 cc. per minute.

In addition to this blood which drains from the venous side of the coronary system, there is another possible source of blood in the by-passed heart, and this may account for a considerable volume which must be dealt with during the period of open cardiotomy. A reflux through incompetent aortic valves is this second possible source of blood in the cardiac chambers after the caval streams have been diverted entirely into an extracorporeal system. A relative incompetence may be caused by displacement of the heart during cardiotomy, or there may be an absolute incompetence of the valve coincident with other cardiac abnormalities. The authors believe that it is essential that a system be employed which is capable of handling such large volumes and returning them to the extracorporeal circuit without delay.

* From the Department of Surgery and the Department of Pediatrics, College of Medicine of the University of Cincinnati, Cincinnati, Ohio, and from the Fels Institute for Research in Human Development, Antioch College, Yellow Springs, Ohio. This project was supported by grants from the U. S. Department of Health, Education and Welfare, the Playtex Park Research Foundation, the American Heart Association, the Cincinnati Children's Heart Association, and The Crosley Foundation.

** The authors wish to express their [illegible]
of Dr Joe Miller, Miss Ruth Parker, M [illegible]
Helmsworth The Upjohn Company, K [illegible]
in these experiments

$$\frac{dv}{dt} = \frac{dv}{dt} + v\,\Delta v = \frac{dv}{dt} + \tfrac{1}{2}\Delta\,(v\,v - vx \operatorname{curl} v) = \frac{dv}{dt} + \tfrac{1}{2}\nabla\,(v)^2 - 2\,vxw$$

also the rate of change of the scalar property such as density at x, y, z at time ti results in:

$$\frac{d\rho}{dt} = \frac{d\rho}{dt} + v \text{ gradient } \rho$$

since continuity has it that:

$$\frac{d\rho}{dt} = -div.\,(\rho v)$$

then:

$$\left(\frac{d\rho}{dt}\right) = -div\ (\rho v) + v \text{ grad } \rho \approx -\rho\, div.\, v$$

The approximate boundary conditions in the blood channel are such that the velocity is tangential to the bounding surfaces. When the viscosity is considered, the blood moves next to the surface and follows its contours. If the viscosity remains fairly constant, the blood slips along the silastic surface without appreciable friction so that a finite tangential component on the channel surface is allowed. Then the flow integral is.

$$\int_1^2 v\ da = \int_1^2 v\ (dsxk) = \int_1^2 (vxds)\ k =$$

$$= \int_1^2 (vxdy - vydx) = \int_1^2 d\phi = \phi_2 - \phi_1$$

or total flow of fluid along the planes z = 0 and z = 1 and defined by flow lines 1 and 2 is the difference between values ϕ_2 and ϕ_1 of the flow function

SUMMARY

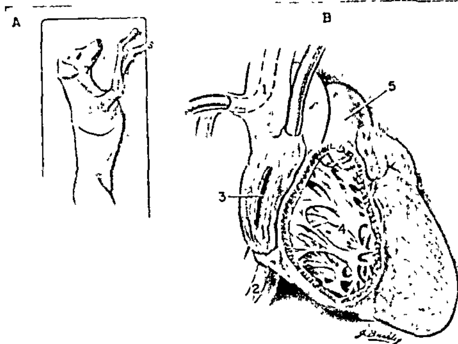
A dual acting blood pump for parabiotic or cross transfusion procedures is described. The nature of blood flow in this apparatus is considered.

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plastic tube which led with a downward slope* into a reservoir (Fig. 2). The funnel opening which presented in the pleural space was guarded by several different means (Lucite balls, stainless steel screen, etc.). This precaution was necessary because without it the left lung would frequently fall against the instrument and partially or completely occlude the opening.

blood permitted the entrance of some air into the funnel line, but this did not result in an intimate mixture of air with blood. A relatively small coalescent surface at the outer end of the funnel line served to eliminate any large



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bubbles which came through the plastic tubing. After passing over this surface the blood collected in a reservoir, and from this chamber it was pumped into the main circuit of the extracorporeal system.

RESULTS AND CONCLUSIONS

Gravity flow of coronary venous blood from open cardiac chambers into this pleural space drainage system has proven to be a satisfactory method. The relatively large volume of blood, as shown in Table 1, is conducted rapidly into a reservoir from which it is drawn into the extracorporeal circuit. Use of this method prevents loss of coronary venous blood, and

* The inner diameter of the Tygon tubing used to connect the funnel with the reservoir was 6 mm The funnel end of the pleural space drainage system was 25 cm. higher than the reservoir.

There is a third source of blood in the cardiac chambers, even after the conditions for total by-pass have been established. This is the bronchial artery flow which, after coursing through the pulmonary bed, must be permitted to drain into the left auricle. This has been a relatively small volume in the conditions of these perfusions in normal animals. However, the well-known enlargement of these channels in association with certain congenital cardiovascular anomalies[3] may significantly increase the volumes to be dealt with from this source.

METHODS

Initially, the authors employed a simple suction system to withdraw these rather large volumes from the chambers after the by-passed heart had been opened The excessive hazard of air embolism inherent in this method was not recognized until after a long series of total perfusions had resulted in the death of the recipient animals. The sequence which resulted in air embolism was as follows It was inevitable that some air would be drawn into such an aspirating line along with the blood. This air-blood mixture was passed through a coalescence chamber identical with the one employed in the principal circuit of the extracorporeal system.[4] Transit through that chamber produced coalescence of the air bubbles and these came away from the surface of the blood during the short period that it collected in the reservoir It was assumed from the macroscopic appearance of the stream of blood as it emerged from that chamber that there was no more air included which would flow into the animal as gas emboli. The error in this assumption was recognized only after a series of experiments in which all of the other possible sources of gas emboli were eliminated and lethal air embolization was still demonstrable in the dog's cerebral arterial system The air sucked in this aspirator, along with the coronary blood, was finally incriminated as the source of the emboli. The failure of the coalescence chamber to produce coalescence of all of the finest air bubbles was recognized.* *Methods employing a somewhat different principle were then considered*

A selective sucker was considered as a possible solution to the problem. This type of instrument is one that would draw up only liquid and then turn off when no more is at the tip. A different method was chosen for trial, however, because the mechanical factors involved were simpler than those anticipated with the intermittent suction device. In this new method, both auricles and the right ventricle of the by-passed fibrillating heart† were opened and then the organ was held so that the coronary venous blood gravitated away from the endocardial surface where the optimal exposure was desired (Fig 1). Some shifting of the right lung and the edges of the pericardium prevented the loss of any of this venous blood by spillage over the margins of the thoracotomy incision The blood coursed downward into the dependent left pleural space, and from there it was funneled into a

* The efficiency of the coalescence chamber for the purpose for which it was originally devised, the coalescence of very fine oxygen bubbles, is not open to serious question The authors believe that the coalescence of fine oxygen bubbles is different from the coalescence of fine bubbles of air and the failure of the chamber to handle air in this form was the nucleus of the difficulty.

† It is the authors' routine to induce ventricular fibrillation in the by-passed heart The advantages of *this change in the heart's rhythm will be presented in a separate publication* In more than 65 consecutive experiments defibrillation of the ventricles has been accomplished without difficulty following closure of the incisions in the cardiac chambers

stances of this complication result from incomplete removal of air after closure of the cardiotomy incisions.)

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EXPERIMENTAL EXPOSURE OF THE AORTIC VALVE*

Laboratory Studies and a Clinical Trial

GEORGE H. A. CLOWES, JR., AND WILLIAM E. NEVILLE

An experimental technique has been evolved to permit exposure of the aortic valve in a dry field for a period of time sufficient to correct surgically certain abnormalities of this structure. Although this requires the use of a blood pump and oxygenator, it is not intended to describe this apparatus, which has been fully discussed previously.[1] Rather, it is desired to present in some detail this technique and the results of its use, for it may prove useful with any satisfactory system for the artificial maintenance of circulation.

Although a number upon the aortic valve,[2-5] its ver cesses by which it is affected ma Yet three problems stand in the way of its successful exposure. First, the circulation must be artificially diverted around it or the body must be cooled while the circulation is interrupted[6,7] to avoid serious damage to vital organs. The former method was adopted in this work. Second, the coronary circulation must be interrupted for a period as long as the valve is exposed. It is necessary to determine just how long at the ambient temperature the heart can withstand such acute ischemia and still function normally afterward. Finally, surgical techniques must be devised to accomplish the exposure with a minimum of blood loss, trauma, and delay in returning circulation to the coronary arteries. Studies undertaken to answer these questions are to be described.

METHODS AND MATERIALS

Mongrel dogs weighing 8 to 18 kilograms were used without special preparation in these experiments. To perfuse these animals, venous blood

* From the Department of Surgery, Western Reserve University School of Medicine, Cleveland City Hospital, Cleveland, Ohio. This study was in part supported by a grant from the Cleveland Area Heart Society and aided by the use of equipment acquired through a grant from the Akron District Heart Association.

therefore it is possible to maintain a constant blood volume in the combined systems of the subject animal and the extracorporeal circuit.

This method of gravity flow drainage of coronary venous blood exposes the blood to air for a brief interval. However, there is no mixing of blood with small collections of air to form a system of fine bubbles. The pleural

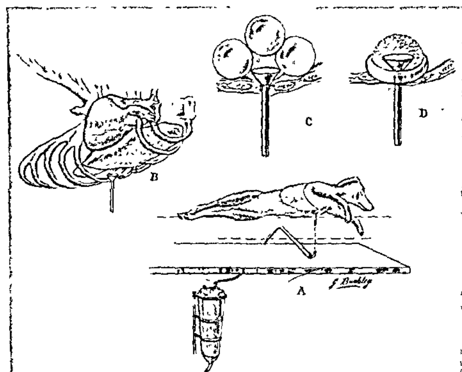


Fig 2 A illustrates the arrangement of the external parts of the pleural space drainage system. B is a cutaway diagram which shows the relation of the funnel end of the drainage system to the pleural surfaces of the dependent hemithorax C and D illustrate the use of Lucite balls or a stainless steel screen as a guard to keep the lung surface away from the funnel

space drainage method, therefore, reduces the possibility of air embolism in those experiments in which the coronary venous flow coming from the opened cardiac chambers must be reinfused into the animals' arteries. Since the adoption of this method the incidence of air embolism in the perfused animal has been reduced by more than 90 per cent. (The remaining in-

*Table 1 Rate of Total Flow and Coronary Flow**

WEIGHT	TOTAL FLOW CC /MIN	CORONARY FLOW CC /MIN.
13.6	1197	209
19 3	1370	268
16 9	1606	189
16 7	1169	210
11 6	1125	147

* Measurements during total by-pass in five experiments which resulted in postopera-

pulmonary artery, which promptly filled the left side of the heart and base of the aorta with blood. With the special clamp closing the incision, the clamp across the aorta near the innominate artery was removed, restoring coronary circulation. The incision was closed with an everting mattress suture of fine silk approximating the edges of the incision which were then oversewn with a second continuous suture.

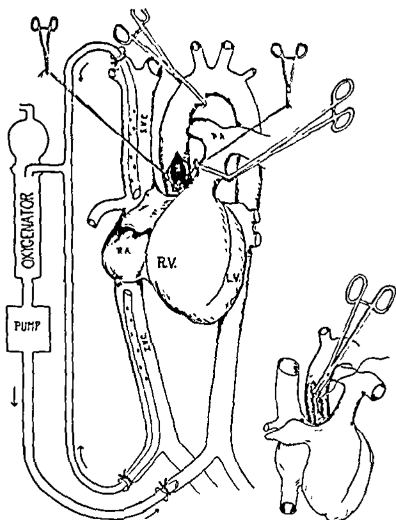


Fig 1 Diagram of the exposure of the aortic valve through an incision in the wall of the aorta Circulation is excluded from this area by a clamp across the pulmonary artery and the aorta itself just proximal to the great vessels to the head The inset shows the application of a special serrated clamp to control the aortic incision while it is being sutured.

After the heart had returned to a good pink color, defibrillation, if necessary, was carried out with one or more shocks No procaine or other drugs were used in this procedure. As normal heart action returned, the pump intake through the venous catheters was gradually reduced until full maintenance of circulation was dependent upon the heart. Protamine was given slowly by intravenous saline drip to the point when clotting time was reduced to less than ten minutes. After complete reexpansion of the lungs, the

was drawn into a pump-oxygenator dependent upon the formation large oxygen bubbles. The oxygenated blood was returned to the arterial system under pressure by either a finger pump or one actuated by air pressure.

The electroencephalogram, electrocardiogram and arterial blood pressure were recorded by a Grass ink writer oscillograph. Defibrillation of the ventricles was effected by a defibrillator which delivered 110 volts A.C.* Shocks of approximately one-fifth second were used.

Arterial blood oxygen and carbon dioxide content was determined by Van Slyke analysis. A Cambridge glass electrode was used to measure pH. The carbon dioxide tension was estimated by the nomogram of Singer and Hastings.[8]

OPERATIVE PROCEDURE

Under Nembutal anesthesia (60 mg. per kilogram of body weight) animals were placed in a supine position. The chest was entered through an incision in the third right interspace which was extended across the sternum into the left chest cavity. This afforded an excellent view of the great vessels and the base of the heart. The pericardial incision was made over the right ventricle and carried up to the upper limit of the pericardium. The aorta was dissected free of the pulmonary artery and cleared of adventitial tissue as far as the innominate artery. Complete dissection to free it of fatty tissue at the base of the heart at its origin required retraction of the pulmonary artery to the left and the right auricular appendix to the right side.

When the dissection had been finished and complete hemostasis attained, heparin (5 mg per kilogram of body weight) was administered intravenously. As shown diagrammatically in Figure 1, the pump-oxygenator was connected by catheters placed in the venae cavae through an external jugular and a femoral vein, and by a cannula placed in the femoral artery directed centrally Being previously filled with heparinized donor blood, the apparatus was turned on and partial perfusion started

Two fine silk stay sutures were placed in the base of the aorta 1 to 2 cm. from the heart Traction on these tented the anterior wall of the aorta, permitting placement of the specially designed serrated clamp (Fig. 2) so that its turned-up tip rested firmly against the heart. This permitted making a 2 5 cm. incision in the aortic wall while only partially occluding its lumen, as shown in the inset of Figure 1 A Satinsky clamp was then placed across the pulmonary artery to prevent blood from entering the lungs and the left heart, thus diverting the whole circulation to the pump-oxygenator. After 2 minutes to allow for the lungs and left heart to empty themselves, a clamp was placed across the aorta just proximal to the innominate artery. This excluded the aortic valve and coronary arteries from the circulation, while perfusion continued to the remainder of the body

Removal of the special aortic clamp and sucking out of a small amount of residual blood permitted an excellent view of the aortic valve and the coronary orifices when a small thyroid pole retractor was placed in the lower end of the aortic incision.

To close the incision the special aortic clamp was put in position aided by traction on the two stay sutures. However, it was not closed until all air had been evacuated from the left ventricle by removal of the clamp on the

* Kindly furnished by the H J Rand Foundation, Cleveland.

of suture line described in the operative procedure was the best of the various methods tried.

In the second series nine of the ten animals survived until sacrificed. One died of undetermined causes 16 hours postoperatively. The temperature fell from 3° to 5° C. during the operation.

Data on the ability to defibrillate the heart and on survival of animals following varying periods of acute myocardial ischemia are given in Table 1.

All of the experimental animals included in Table 1 showed the normal electroencephalographic pattern of barbiturate anesthesia throughout the perfusions.[9] The electrocardiogram showed certain characteristic changes when the animal was subjected to acute ischemia. Within two minutes signs of A-V block usually appeared. Ventricular extrasystoles occurred. This was accompanied by severe depression of the S-T segment. At five minutes arrest usually was present, to be shortly followed by the irregular pattern of ventricular fibrillation. In the protracted experiments of 25 minutes or more the potential was greatly decreased prior to restoration of circulation to the coronaries. As the coronary circulation was resumed and the heart became a good pink color, the fibrillation became active. Following defibrillation an

Table 1. The Effects of Acute Myocardial Ischemia on Defibrillation of the Heart and upon Survival

	LENGTH OF ACUTE MYOCARDIAL ISCHEMIA (Minutes)				
	5	10	25	35	45
Number of experiments	2	10	2	3	2
Number of ventricular fibrillations	1	10	2	1	0
Number successfully defibrillated	1	10	2	1	0
Number of survivors	2	9	2	0	0

A-V partial block was often present, but a normal sinus mechanism usually returned within a few minutes.

As may be seen, only one dog was successfully defibrillated after periods of more than 25 minutes of coronary occlusion. In this instance death occurred six hours later.

CLINICAL EXPERIENCE

G P., U-1459, was a 55 year old white man in intractable cardiac failure with a diagnosis of severe aortic stenosis, moderate mitral stenosis and regurgitation, and hemiparesis from a previous cerebral infarct. Under extradural block with an endotracheal tube in place, the operative procedure as described above was carried out. Shortly after induction of anesthesia he suddenly became unconscious and the blood pressure fell from 110 to 65 mm. Hg. The blood pressure responded to a vasopressor drug. He was connected to a large model type I oxygenator.[1] The aortic valve was exposed and the posterior commissure was incised. In all, the coronary circulation was interrupted for six minutes. The heart did not stop beating and the electrocardiogram showed only a depression of the S-T segment. When circulation was restored to the coronary arteries with reapplication of the special aortic clamp, the heart promptly became pink and resumed a forceful beat. When the pump was shut off nine minutes later the heart dilated

pericardium and chest were closed, leaving a drainage tube under slight negative pressure until the animal was conscious. Procaine penicillin, 300,000 units, was given daily for one week.

EXPERIMENTAL PROCEDURES

The operation described above was performed on 26 animals. The incision was maintained open for periods ranging from five minutes to 45 minutes. Electrocardiograms, electroencephalograms, and arterial blood pressure were recorded at intervals throughout the procedures. Arterial blood samples were taken before, during, and after the perfusions to determine whether the pump-oxygenator had been functioning well in maintaining normal pCO_2 and O_2 saturation. No attempt was made to raise or lower the animals' body temperature.

The experiments were divided into three series. In the first eight the aortic incision was open for five minutes. The second series, done with strict atten-

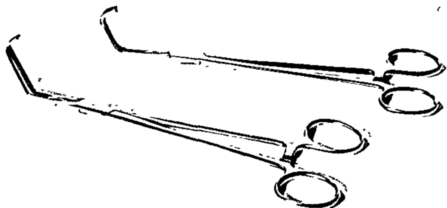


Fig 2 Photograph of two sizes of the special serrated clamp for holding the aortic incision Its use permits immediate restoration of coronary circulation after exposure of the aortic valve.

tion to asepsis, were kept open for ten minutes. Finally, to test the length of time the myocardium could withstand acute ischemia and still return to normal function, a third series of eight experiments was undertaken in which the aortic incision was left open for 25 minutes in two, 35 minutes in three, and 45 minutes in three. Only data from those experiments in which the oxygenation and carbon dioxide removal was adequate during a good perfusion were included for analysis of this problem. In determining the ability to survive after defibrillation of the heart following various lengths of acute ischemia, no animals were included which obviously died of hemorrhage.

RESULTS

Of the first series of eight animals only two survived. One died when the pump failed. Two died of minute fibrin emboli produced in the oxygenator. Three died of hemorrhage from the aortic incision. This experience indicated that control of the aortic incision by the specal clamp and the type

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HEMODYNAMIC EFFECTS OF EXPERIMENTAL DRAINAGE OF PULMONARY VEINS TO RIGHT ATRIUM*

DONALD E. BOWES, H. J. C. SWAN, AND JOHN W. KIRKLIN

The term "anomalous pulmonary venous connection" recently has been proposed to designate the congenital anatomic anomaly in which a venous pathway from either or both lungs fails to enter directly the left atrium, but instead connects with the right atrium or one of its tributaries.[1] The term "anomalous pulmonary venous drainage," which has a physiologic or functional connotation, implies that, although the venous connections may or may not be normal, some or all of the arterialized pulmonary venous blood recirculates through the right atrium–pulmonary artery circulation instead of passing by way of the left ventricle to the systemic arterial circulation.[2] The introduction of this distinction was occasioned by the demonstration in a human case of atrial septal defect of total drainage of the blood from the right lung into the right atrium.[2]

This paper is a report of a method for the creation of an anomalous pulmonary venous connection in the dog by means of which the veins draining the apical and cardiac lobes of the right lung were caused to empty into the right atrium. The hemodynamic effects of such lesions were studied 3 to 30 weeks following operation, and the nature of the anomalous connection was ascertained by examination post mortem.

METHODS

Surgical. Mongrel dogs weighing 17 to 20 kg. were anesthetized with sodium pentobarbital. Access to the interior of the right atrium was possible by the atrial well technique of Gross.[3] The prominence of the intervenous tubercle (limbus of fossa ovalis) was identified and incised, creating a com-

* Abridgment of thesis submitted by Dr. Bowes to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Master of Science in Surgery.

and went into arrest followed by fibrillation The pump was again started. The heart was defibrillated The pump intake was gradually reduced, and 51 minutes later was shut off when the heart appeared able to maintain the circulation Protamine was administered, and the chest was closed. Spontaneous respiration did not reappear, and the heart stopped beating one hour later. Autopsy revealed not only the incised commissure of the aortic valve, but a 0.4 cm. stab wound which had been made in the medial cusp which had perforated the ring. In addition, an old infarct was found in the brain but no fresh one. The lungs were congested, with a fresh infarct being present.

DISCUSSION

The work of Wesolowski and his associates[10] showed that the survival rate of animals subjected to acute myocardial ischemia for periods of 30 minutes and 60 minutes was quite low even if the ventricle had been effectively defibrillated. However, the excellent recovery rate in those animals of this series subjected to 25 minutes or less of acute coronary occlusion made it seem reasonable to attempt the cure of a desperately ill patient

It is apparent that the normal dog heart probably does not suffer serious damage at the temperatures present in these experiments for periods up to 25 minutes The patient's heart deprived of circulation, but performing no work, hardly changed the character of its potential discharge as reflected in the cardiogram during a period of six minutes. The cause of the failure following surgery is hard to explain, for there was no discoverable evidence of air embolism in the coronaries Further, it appeared inconceivable that the small perforation of the ring could have produced a right to left shunt of any importance.

Therefore, it appears that this technique may be suitable for surgical procedures of relatively short duration with the use of an artificial means of maintaining circulation Indeed, the normal dog heart seems to withstand periods of acute coronary occlusion up to 25 minutes.

SUMMARY

1. A technique is described for exposure of the aortic valve to direct vision while the circulation to the remainder of the body is maintained by an artificial method of oxygenating and pumping blood.

2. In a series of ten dogs subjected to this procedure, nine survived.

3. Studies to determine the length of time the normal dog heart can withstand acute ischemia suggest that normal function can be expected to return if circulation is restored to the coronary arteries within 25 minutes.

4. The application of this technique to one clinical case is described.

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Hemodynamic Studies. A minimal period of 3 weeks was allowed for the animal to recover following production of the defect. Cardiac catheterization was carried out with each animal under sodium pentobarbital anesthesia, using the techniques described by Wood.[4] Intracardiac and arterial pressures were recorded by means of strain-gauge manometers, and the oxygen saturation of blood samples drawn from the chambers of the heart and great vessels was determined by photometric[5] and manometric methods.[6]

Values for pulmonary and systemic flow were determined by the Fick principle while the animal breathed 100 per cent oxygen. During the measurement of oxygen consumption, blood samples were drawn simultaneously from the femoral artery, the pulmonary artery and the inferior vena cava, and shortly thereafter from the superior vena cava. The oxygen content of these samples was estimated by the method of Van Slyke.[6] Pulmonary ($F_{\bar{p}}$) and systemic ($F_{\bar{s}}$) flows were determined according to the formulas:

$$F_p = \frac{O_2 \text{ conc (ml/min)}}{Q_{O_2}\text{ P.V.} - Q_{O_2}\text{ P.A.}},\quad F_{\bar{s}} = \frac{O_2 \text{ conc (ml/min)}}{Q_{O_2}\text{ P.V.} - Q_{O_2}\text{ M.V.B.}},$$

Where Q_{O_2} P.V. = oxygen content of pulmonary vein blood (cc./liter).

Q_{O_2} P.A. = oxygen content of pulmonary artery blood (cc./liter) and

Q_{O_2} M.V.B. = oxygen content of mixed venous blood (cc./liter).

The last value was calculated from the relation

$$Q_{O_2}\text{ M.V.B.} = \frac{2\,(O_2 \text{ sat'n of I.V.C. blood}) + O_2 \text{ sat'n of S.V.C. blood})}{3}$$

$\times$ O_2 capacity

Indicator dilution curves were recorded by a cuvette oximeter through which the femoral artery blood was permitted to flow, following the injection of T-1824 into the chambers of the heart and great vessels, as described by Swan and Wood.[7] Such curves indicate the nature of the vascular pathway *from the site of injection to the site of sampling.*

RESULTS

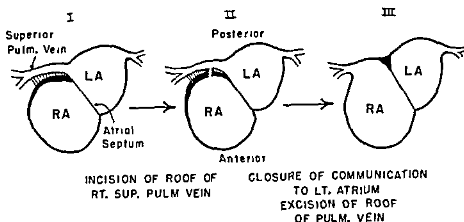
The procedure was successfully completed in 16 of 21 dogs subjected to operation. Those dogs which survived operation had an uneventful postoperative course. Heart murmurs did not develop in any and none appeared incapacitated in any way.

When cardiac catheterization was carried out on the 16 dogs, the data obtained indicated that an unexpected atrial septal defect existed in 4 of the animals. Hence the results for these animals are considered separately.

Observations on 12 Dogs with Anomalous Connection of the Right Superior Pulmonary Vein. The average values for intracardiac pressures are given in Table 1, along with the values for a group of normal animals studied under identical conditions, for comparison.

The average values for the experimental group did not differ significantly from those for the normal dogs. In respect to the animals with anomalous connections, the contours and absolute pressures for the pulmonary artery wedge and pulmonary vein wedge were similar but not identical in magnitude. The normality of these pressures contrasts with the intracardiac

munication between right and left atria (Fig. 1). The free posterior edge of the interatrial septum was then sutured to the posterior rim of the left atrial orifice of the right superior pulmonary vein, this closed the interatrial com-



munication but allowed the right superior pulmonary vein to connect directly with the right atrium The orifice of this communication was enlarged by removing the ventral wall of the right superior pulmonary vein and the

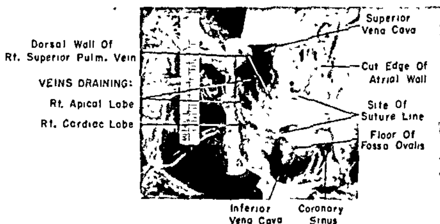


Fig. 2 Interior of the right atrium of a dog weighing 18 kg, killed 3 months after surgical production of anomalous connection of the veins draining the apical and cardiac lobes of the right lung. The unobstructed orifices of the anomalously draining veins are seen at the lateral margin of the right atrium Note the small depressions in the floor of the right atrium which are the only residual scarring, and which occur at the points of suture in the atrial wall.

adherent dorsal wall of the right atrium (Fig 2). This procedure resulted in the right apical and cardiac lobe veins communicating with the posterolateral wall of the right atrium through an orifice which was found to measure 1.0 to 2 5 cm. in its principal diameter.

found to be practically the same as the average value for a group of normal dogs, in spite of a wide range of values. The pulmonary flow exceeded the systemic flow by 1.1 liters/minute, owing to the presence of a left-to-right shunt averaging 21 per cent of the pulmonary flow. A wide variability of values was obtained (0 to 50 per cent of pulmonary flow) but a range of 15 per cent to 35 per cent of pulmonary flow was found to include 11 of the 17 observations.

Circulatory Dilution of Injected Dye. If indicator is injected into the right or left main pulmonary arteries or branches thereof, the differences in vascular pathway from each injection site will determine to a large extent the

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artery on the one hand and, on the other, the cardiac and apical lobe branches of the right pulmonary artery (Fig. 3*a*). The basic patterns seen in this figure are typical of the curves obtained in the other animals. When dye is injected into the artery supplying a division of the right lung which drains anomalously, the dye curve differs from normal in that the instant of first appearance of dye at the site of sampling from the femoral artery is delayed. Furthermore, the deflection obtained is moderately reduced and the return of concentration is somewhat prolonged. The delay in the first appearance of dye is related to the longer circulatory path which the dye has to traverse to reach the sampling site, and the changes in the contour of the dilution curve are related to the presence of a moderate left-to-right shunt. When dye is injected at positions proximal to the artery which supplies the anomalously draining lobe, the resultant dilution curve may reflect the differing nature of the pathways which it has to traverse to reach the sampling site. When dye passes only to a lobe the veins of which drain directly into the left atrium, normal dilution curves are obtained.

Observations on 4 Dogs with Anomalous Connection of the Right Superior Pulmonary Vein and, also, Unexpected Small Atrial Septal Defects. As has been said, when these animals were studied by cardiac catheterization, certain abnormalities of the dye-dilution curves indicated that a small atrial septal defect existed in 4 dogs. In one of these animals the catheter passed through the atrial septal defect into the left lung.

In 2 of these dogs the appearance time of the dilution curve recorded from the femoral artery, when dye was injected into the inferior vena cava (Fig. 3*b*), was shorter than the appearance time when dye was injected into the right ventricle, and a definite break was seen on the initial limb of the curve. These features of the dilution curve indicated the presence of an interatrial communication through which a right-to-left shunt was occurring.

In all 4 of these animals the dilution curve recorded following injection of dye into the artery which supplied the anomalously draining lobe indicated the presence of an atrial septal defect. In contrast to the curves obtained from this site in the first group of 12 animals, in these 4 animals there was a break on the initial portion of the curve due to the early arrival of a small amount of dye at the recording site. This small peak of concentration was coincident in time with the major peak of concentration seen for the dilution curves recorded when dye was injected into the arteries supplying normally draining lobes, and indicated that some dye from the anomalously

pressures in dogs with large atrial septal defects, in which a rise in all the intracardiac pressures have been demonstrated.[8]

The oxygen saturation of blood samples from the right atrium, and from the superior and inferior venae cavae, was determined by means of a cuvette oximeter—an instrument of high relative accuracy (Table 2). In

Table 1 Values for Intracardiac and Pulmonary Pressures in Normal Dogs and in Dogs with Anomalous Connection of the Right Apical and Cardiac Lobe Veins

		PRESSURE (MM. HG)					
		PULMONARY ARTERY					
STATUS OF ANIMALS	NO OF ANIMALS	WEDGE	PERIPHERAL	MAIN	RIGHT VENTRICLE	RIGHT ATRIUM	PULMONARY VEIN WEDGE
Normal	13	7/4	24/11	26/10	27/–1	3/–1	——
Anomalous connection of right superior pulmonary vein	12	10/6	23/11	27/12	29/1	6/2	21/15

each animal, the oxygen saturation of the blood samples drawn from the superior vena cava exceeded the saturation of the sample from the inferior vena cava For the group, this difference averaged 9 per cent while the animals were breathing air. In 8 of the animals the oxygen saturation of blood samples drawn from the right atrium exceeded the saturation of the superior vena caval blood, this demonstrates that blood of high saturation

Table 2 Oxygen Saturation Values of Blood Obtained from Different Locations in the Heart and Great Vessels, the Values for Pulmonary and Systemic Blood Flow and the Magnitude of the Left-to-right [illegible] Connection of Right [illegible] Veins

	OXYGEN SATURATION (VOL PER CENT)					BLOOD FLOW		
ANIMAL BREATHING	PULMONARY ARTERY	MIXED VENOUS BLOOD*	SUPERIOR VENA CAVA	RIGHT ATRIUM	INFERIOR VENA CAVA	PULMONARY L/MIN	SYSTEMIC L/MIN	LEFT-TO-RIGHT SHUNT, PER CENT OF THE PULMONARY FLOW
Air	82	80	86	92	77	——	——	——
Oxygen	96	91	90	95	88	4.5 (6.3-2.9)‡	3.4† (5.4-1.5)‡	24 (0-50)‡

* Calculated from inferior and superior caval samples, see text for details
† Normal values for the dog, 3.2 L/min
‡ The numerals in parentheses indicate the extreme values

entered the right atrium. In the remaining 4 dogs, the oxygen saturation of right atrial blood was equal to or less than the saturation of superior caval blood. In each animal it was possible to obtain blood samples of widely [illegible] within the right atrium, [illegible] from the lateral region [illegible]), the average value was

found to be practically the same as the average value for a group of normal dogs, in spite of a wide range of values. The pulmonary flow exceeded the systemic flow by 1.1 liters/minute, owing to the presence of a left-to-right shunt averaging 24 per cent of the pulmonary flow. A wide variability of values was obtained (0 to 50 per cent of pulmonary flow) but a range of 15 per cent to 35 per cent of pulmonary flow was found to include 11 of the 17 observations.

Circulatory Dilution of Injected Dye. If indicator is injected into the right or left main pulmonary arteries or branches thereof, the differences in vascular pathway from each injection site will determine to a large extent the differences in the resulting pattern of arterial dilution. Important differences have been found between the curves following injections of dye into the left pulmonary artery and the diaphragmatic branch of the right pulmonary artery on the one hand and, on the other, the cardiac and apical lobe branches of the right pulmonary artery (Fig. 3*a*). The basic patterns seen in this figure are typical of the curves obtained in the other animals. When dye is injected into the artery supplying a division of the right lung which drains anomalously, the dye curve differs from normal in that the instant of first appearance of dye at the site of sampling from the femoral artery is delayed Furthermore, the deflection obtained is moderately reduced and the return of concentration is somewhat prolonged. The delay in the first appearance of dye is related to the longer circulatory path which the dye has to traverse to reach the sampling site, and the changes in the contour of the dilution curve are related to the presence of a moderate left-to-right shunt. When dye is injected at positions proximal to the artery which supplies the anomalously draining lobe, the resultant dilution curve may reflect the differing nature of the pathways which it has to traverse to reach the sampling site. When dye passes only to a lobe the veins of which drain directly into the left atrium, normal dilution curves are obtained.

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[illegible] arteries supplying the anomalously

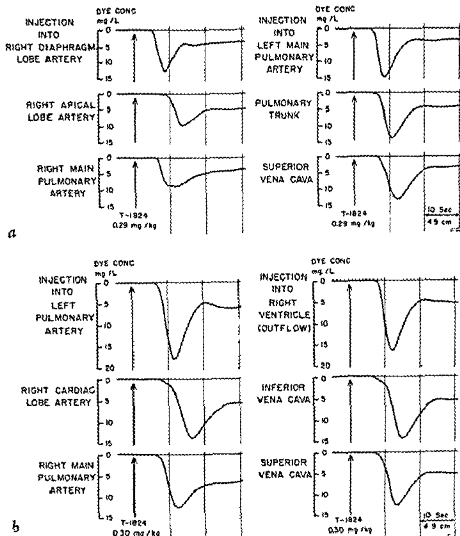


Fig 3 Demonstration by indicator dilution curves recorded at the femoral artery following injection of T-1824 into the pulmonary arteries and venae cavae *a*, Dog weighing 17 kg Anomalous pulmonary venous drainage of blood from the apical and cardiac lobes of the right lung *b*, Dog weighing 16 kg Same anomalous drainage and, in addition, a small interatrial communication

a, Curve normal following injection into the right diaphragmatic lobe artery and the left pulmonary artery. The curve recorded following injection into the apical lobe represents a long appearance time because of the long vascular pathway traversed by the dyed blood, which must recirculate through the lungs before it reaches the recording instrument, the changes in contour of the disappearance slope are due to a moderate left-to-right shunt The first peak in the right pulmonary artery curve is due to dye traversing the normal vascular pathway via the right diaphragmatic lobe, the second peak is due to dye returning to the right atrium via the right apical and cardiac lobes and recirculating before reaching the recording instrument The curves recorded following injection into the main pulmonary artery and superior vena cava are consistent with a small left-to-right shunt.

b, Curve normal following injection into the right diaphragmatic lobe artery and the left pulmonary artery Certain unusual features not evident in the majority of the curves are seen in the dilution curves recorded relative to this animal Note the small break in the initial part of the curve recorded following injection into the artery of the anomalously draining right cardiac lobe This initial deflection coincides in time with the

connected lobe was passing directly to the recording site without recirculating through the lungs. This finding strongly suggested that an interatrial communication existed in these animals. Apart from the dye-dilution curves, no other hemodynamic differences from the dogs without defects could be demonstrated. At necropsy a small communication at the caudal margin of the transposed portion of the limbus of the fossa ovalis was demonstrated in each animal.

COMMENT

Satisfactory preparations were made in 12 of the 16 animals which survived the operative procedure. The majority of the animals which died at, or shortly after, operation were among the first animals subjected to operation. Donald, Kirklin, Ellis and Grindlay,[9] using the atrial well technique, created and repaired atrial septal defects in dogs. In one of their experiments they repaired the septal defect by anchoring the septal remnant to the left atrial margin of the right superior pulmonary vein, so that this vein drained, by means of a small opening, into the right atrium. This mishap suggested the method of production of anomalous pulmonary venous connection used in this study.

The technique appears to have advantages over the methods of Hanlon and Blalock[10] and of Gerbode, Yee and Rundle,[11] in which direct anastomosis of veins to the right atrium was carried out. The present technique does not necessitate interruption of the circulation through a portion of the lung, and lessens the possibility of the occurrence of edema or thrombosis of the anomalously connected lung or its veins. Further, absence of an anastomotic junction eliminates the possibility of stenosis of the atrial orifice of a transposed vein. The only sutures in the heart are located at a distance from the opening of the superior pulmonary vein into the chamber of the right atrium.

Hemodynamic studies revealed left-to-right shunts of variable magnitude. However, use of indicator dilution techniques allowed for the demonstration of the abnormal circulatory pathway from the apical and cardiac lobes of the right lung in all dogs. In 4 of the dogs the abnormal dilution curves indicated the presence of small interatrial communications which later were demonstrated at necropsy.

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[illegible] communication.

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CINEMATOGRAPHIC DEMONSTRATION OF VALVULAR DISORDERS OF THE HEART*

KARL P. KLASSEN AND CHARLES V. MECKSTROTH

The static and dynamic anatomy and pathology of the valves of the heart have become of utmost importance because of the tremendous strides in intracardiac surgery. It is extremely difficult to visualize the normal function of these valves when studied at the autopsy or anatomy table in the conventional manner. Under these latter conditions the valves are merely structures of fibrous and muscular tissue of varying length and thickness but without animation. Accordingly the authors have devised a simple method of simulating the actual dynamics of the heart valves of postmortem specimens to provide the pathologist, clinician and surgeon with a visual demonstration of the normal or altered premortem action of these valves.

The method to be described was first used to demonstrate the mechanism of the normal (Fig 1) and abnormal mitral valve in fresh, non-Formalin-fixed hearts Using variations of the same technique the other heart valves (Figs 2 and 3) have been visualized and studied To capture this motion, cinematography has been used to its best advantage and the results have produced excellent teaching material for not only the medical students, interns and residents, but physicians in general

METHOD

The principle consists of delivering a constant amount of tap water through rubber tubing into the right or left ventricle depending upon the valve to be studied (Fig 4). A rubber tube of larger caliber is also placed inside the ventricle, and intermittent closure of this outlet provides the increase of interventricular pressure necessary to open or close the various

* From The Division of Thoracic Surgery, Department of Surgery, The Ohio State University Health Center, Columbus This study was aided by the George Morris Curtis Research Fund.

valves. Two holes are cut in the side of the outlet tube to provide additional drainage and assure against complete obstruction by muscular bundles. Opening of the mitral and tricuspid valves is produced by the weight of the column of water above them.

For visualizing the tricuspid valve the inlet rubber tubing of ⅝ inch O.D., ⅜ inch I.D. is placed down the pulmonary artery through the valve



Fig 1. Normal mitral valve in systole and diastole. Photograph of 16 mm movie film.

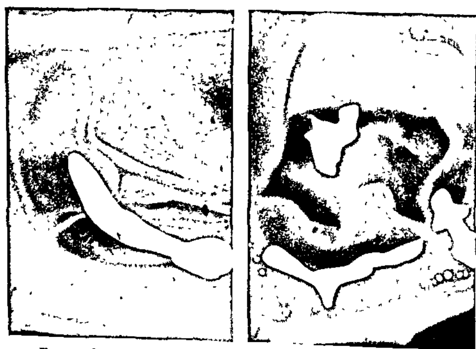


Fig. 2. Photograph of normal pulmonary valve in diastole and systole.

and into the right ventricle. A purse-string suture around the pulmonary artery with several bites through the tubing holds it in place. The outlet tubing measuring ¾ inch O.D., ½ inch I D. is placed through a stab wound at the apex of the right ventricle, being sure not to injure the delicate chordae tendineae of the tricuspid valve. A deep purse-string suture incorporating the myocardium and bits of tubing is used to insure a reasonably tight fit. The superolateral aspect of the right atrium is then removed and the valves are ready for visualization. It is convenient to suspend the heart by stay sutures applied to four corners of the atrium (Fig. 5).

To visualize the pulmonary valve the inlet rubber tubing is placed through the right atrium via the superior vena cava and is held in place with



Fig 3 Stenotic aortic valve in systole and diastole Photograph of 16 mm movie film

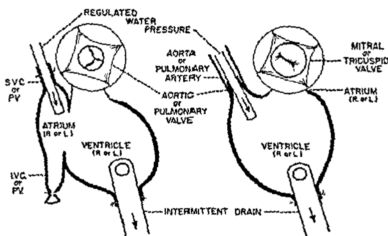


Fig. 4. Diagram of heart preparation for the demonstration of valvular function.

a gathering purse-string suture incorporating most of the atrium. The outlet tubing is identical to that for visualizing the tricuspid valve. At least ½ to 1 cm. of pulmonary artery must be retained distal to the pulmonary valve to provide a column of water of sufficient weight to close the valve during simulated diastole.

Visualization of the mitral valve is similar to that of the tricuspid, with the intake tubing coming through the aorta and past the aortic valve. The outlet in the left ventricle is similar to that of the right, with particular attention given to the placing of the tubing in respect to chordae tendineae.

The aortic valve is animated by placing the intake tubing through the left atrium and mitral valve. The outlet is similar to that for visualization of the

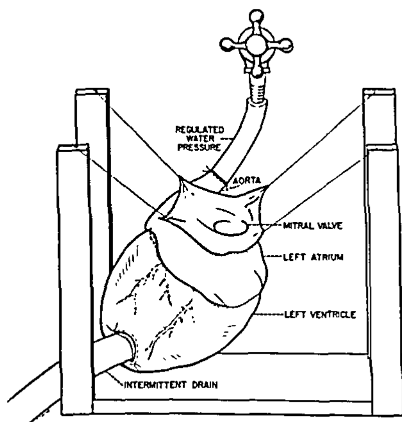


Fig 5. Drawing of heart preparation for the demonstration of mitral valve function.

mitral valve. The pulmonary veins usually must be ligated to minimize leakage.

The valves are animated by supplying a steady amount of tap water to the inlet rubber tubing and by intermittently closing off the outlet tubing with finger pressure. Closing of the outlet tubing simulates ventricular systole whereas opening simulates diastole. In this way the heart can be made to function at any desired rate and output. Quantitative measurements of stroke volume can be carried out.

This simplified method of valve visualization has become routine in the postmortem examination of all cases where valvular disease is suspected. Pathologists and students alike are afforded a qualitative view of the pathologic conditions of tricuspid insufficiency, pulmonary stenosis and insuffi-

ciency. If true pathology is scarce, circumferential sutures of fine steel wire can be used to simulate insufficiency while fine silk will serve for stenosis.

In a similar fashion the surgical correction of pathologic valves can be evaluated not only from the standpoint of present methods or new techniques but also in determining completeness of the procedure in patients who succumb following surgical attack on the heart valves. The pathology of mitral insufficiency especially can be easily answered with all of the benefits of visualization. Work is in progress for quantitative measurements of valvular function utilizing this technique.

SUMMARY

An extremely simple and practical method of animation of the cardiac valves in autopsy specimens has been described This method is now used routinely in studying specimens suspected of valvular disease in addition to those with known defects. Color photography has been used to record the heart valves in action and the effects of surgical correction on available pathology. It is hoped that the medical student of tomorrow may have a better understanding of valvular action from this new method of teaching.

THE SIMULTANEOUS RECORDING OF BLOOD PRESSURES IN THE LEFT HEART AND AORTA BEFORE AND AFTER MITRAL SURGERY*

AARON HIMMELSTEIN, ALVIN J. GORDON, EUGENE BRAUNWALD, RICHARD LASSER, AND MARK M. RAVITCH

Many investigators have recorded blood pressures on the left side of the heart by various techniques including direct puncture in the operating room and also before and after operative manipulation This usually has been done serially as single tracings and, except in a few instances, has not been reported as simultaneous studies on the same beats. In one of these,[1] rescaling of the tracings was done to compare the same beat as it was recorded in two chambers. Our report is based on the simultaneous recording of pressures from the left side of the heart and aorta with the opportunity that this affords for beat by beat analysis.[2] Since the recordings were made as

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studied immediately after ligation of a patent ductus and one after pneumonectomy, during an episode of nodal rhythm. Eight were patients operated upon for mitral stenosis, of whom seven had a commissurotomy. No bleeding or other difficulties have occurred as a result of these needlings

METHOD

The pressures were photographed from an oscilloscope tube face. The system† incorporated an electron switch so that one electron beam recorded

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† Made by Electronics for Medicine, New York, N. Y.

all three pressures and the electrocardiograph When sharp wave fronts occur, the tracings are seen as dotted lines and the time between dots is known to be 0.0013 sec. This allows of accurate timing during sharp changes in pressure. The method of recording prevents parallax errors of more than (4 × 0 0013) 0 005 secs. Each pressure channel was adjusted to the same sensitivity and to the same zero base line. Puncture of the left auricle was made through the auricular appendage or its stump using a 20 gauge needle 1½ inches long. The left ventricle was punctured in a clear area near the base. The aorta was punctured with a 20 gauge needle, 1 inch long, in some instances just above the valve and in others just beyond the subclavian. All

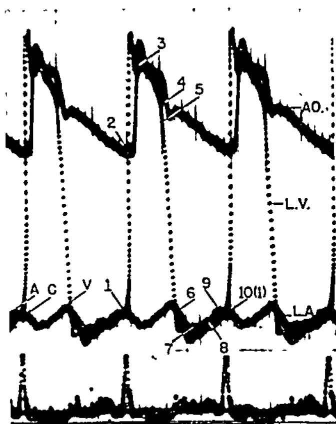


Fig 1. (O W) Simultaneous left auricular, left ventricular and aortic pressures and ECG in a 16-year-old female considered to be a cardiovascular normal. Note the absence of a pressure gradient between the left auricle and left ventricle during diastole.

needles had short bevels and were connected to Statham P23A transducers by 4 feet of heavy-walled vinyl plastic tubing.

RESULTS

The Normal Cardiac Cycle. Figure 1 shows the tracings from O.W., a 16 year old woman who underwent a thoracotomy to remove a thymic cyst. She was considered a cardiovascular normal. The symbols are those used by Wiggers[2] to describe events of the cardiac cycle. Ventricular contraction begins at *1* and virtually simultaneously mitral valve closure occurs, as indicated by the crossover of the auricular and ventricular curves. The "C" wave in the left atrium begins at this time. The isometric contraction period

begins at *1* and ends at *2*, the moment of aortic valve opening, as indicated by the onset of the aortic pressure rise. The period of maximum ejection (*2* to *3*) lasts until the peak of the aortic pressure pulse (*3*) and is followed by the period of reduced ejection (*3* to *4*), which is completed at the beginning of the incisura (*4*), a point sometimes difficult to identify on our

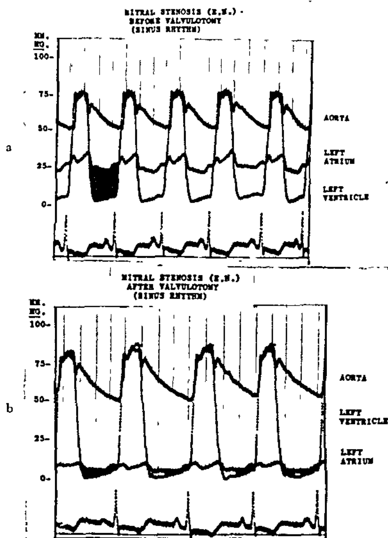


Fig. 2 (E H) Simultaneous left auricular, left ventricular and aortic pressures and ECG in a patient with mitral stenosis and normal sinus rhythm The pressure gradient during diastole has been shaded in one beat of each part of the figure *a*, Before manipulation of the valve. *b*, After manipulation of the valve Note the decrease in the pressure gradient after operation

tracings. This marks the end of ejection and of systole. During protodiastole (*4* to *5*) the pressure in the ventricle and aorta continues to decline. This phase terminates at the moment of aortic valve closure (*5*), i.e., at the bottom of the incisura of the aortic pressure curve Isometric relaxation (*5* to *6*) then follows and ventricular filling begins at the time the mitral valve

reopens, which is indicated by the crossing of the auricular and ventricular pressure curves (6).

The period of rapid early filling, i.e., the diastolic inflow period, is marked by a continuous decline in the auricular pressure curve. Diastasis, or the period of slowed ventricular filling, begins when the auricular pressure

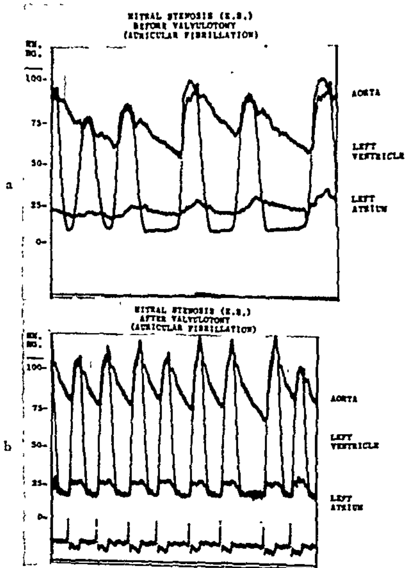


Fig 3 (E S) Simultaneous left auricular, left ventricular and aortic pressures in a patient with auricular fibrillation *a*, Before manipulation of the valve (*there is no ECG on this record*) The paper speed is twice as fast as in the lower part of this figure. *b*, After manipulation of the valve Note that the *pressure gradient* has been reduced to zero but this is due to elevation of the ventricular diastolic pressure

begins to rise during diastole (7). The *onset* of the wave produced by auricular contraction marks the end of diastasis (8). The dynamic interval of auricular systole lasts until the peak of the auricular contraction wave (9), while the inflow phase which follows *ends* at the onset of ventricular isometric contraction (10) and completes the cardiac cycle. Despite the fact that no gradient appears between auricular and ventricular pressures

during diastole, in order for flow to occur a slight gradient must be present.

Mitral Stenosis with Normal Sinus Rhythm. Figure 2a shows the tracings from E H before commissurotomy and 2b after the valve had been opened. This patient had a normal sinus rhythm. The most startling thing on inspection of Figure 2a is the gradient of pressure between the left auricle and

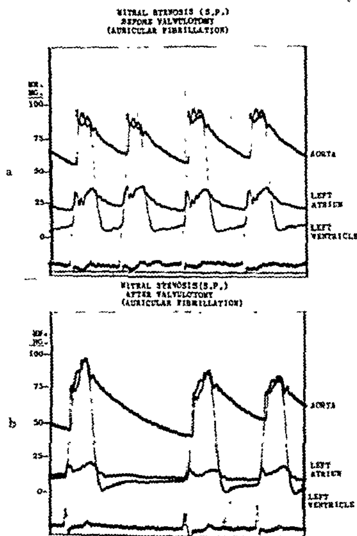


Fig 4 (S P) Simultaneous left auricular, left ventricular and aortic pressure and ECG in a patient with mitral stenosis and auricular fibrillation *a*, *Before manipulation of the valve*. *b*, After manipulation of the valve Note that the longer diastole lasts the closer the ventricular and auricular tracings approach each other and the smaller the pressure gradient becomes

ventricle during diastole (*it has been filled in solid black in one beat*). This is the physiologic representation of the mitral stenosis. The mean pressure in the left auricle is 29 mm Hg In Figure 2b after opening the valve it can readily be seen that the gradient has almost completely disappeared. This reduction to near zero is most marked at the end of diastole. The mean

pressure in the left auricle is now 9 mm. Hg. The reduction to near zero of the pressure gradient between the two chambers is the measure of success in restoring the valve to near normal function, just as the reduction in the gradient between right ventricle and pulmonary artery is the objective evidence of successful operation for isolated valvular pulmonic stenosis. The sequence of events during diastole is now similar to that described in the normal.

Mitral Stenosis with Auricular Fibrillation. Figures 3*a* and *b* are the tracings on E.S. before and after valvulotomy. Figure 3*a* again shows the gradient *across the mitral valve* with the mean pressure in the auricle being 24 mm. Hg. In the tracing taken after opening the valve (Fig. 3*b*), it can be

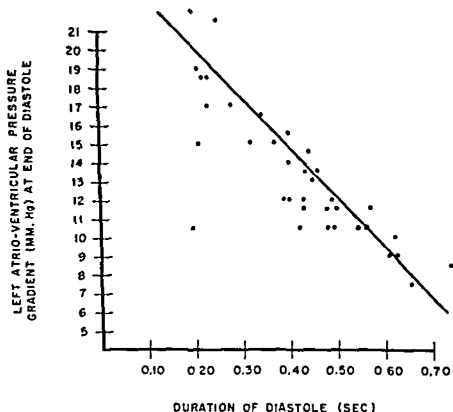


Fig. 5.

seen that the gradient has been completely obliterated. However, the auricular mean pressure is still almost unchanged at 20 mm. Hg. If only pressure tracings in the auricle were available, one might be led to believe that little had been achieved.

Figures 4*a* and *b* are tracings from a patient S.P. before and after operation on the mitral valve. Again attention is directed to the gradient of pressure in diastole before and after operation It has been decreased to a low level after surgery, but inspection of this gradient during various beats shows that the gradient is highest at the beginning of diastole and that when a long diastolic period occurs (as in the first beat of Fig. 4*b*), the gradient decreases continuously.

In Figure 5 we have plotted the gradient at the end of diastole against the total duration of diastole. It brings out the advantage of slow cardiac

rates. Since tachycardia is almost entirely at the expense of diastolic time, it can be seen how this encroaches on cardiac filling with resultant inefficiency, particularly in the presence of an auriculo-ventricular obstruction.

Mitral Insufficiency. In Figure 6*a* are the tracings on S.W. before any manipulation of the mitral valve. The patient had mitral stenosis with minimal insufficiency as determined by the exploring finger. The digital commissurotomy resulted in a rather well appreciated insufficiency, and in Figure 6*b* are the tracings after this had occurred. The change in the form

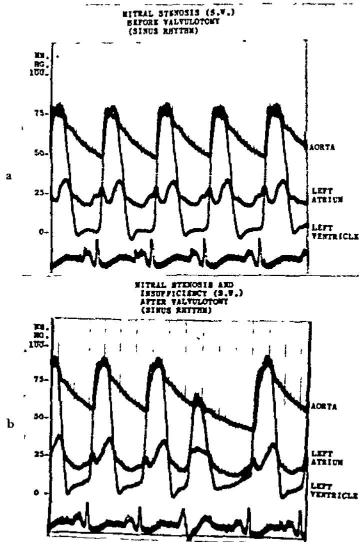


Fig 6 (S.W) Simultaneous left auricular, left ventricular and aortic pressures and

significantly increased in amplitude by the operation, and the rise in pressure in the ventricle at the end of diastole

of the auricular tracing is chiefly marked by the appearance of a rather sharp pressure peak late in systole. This has been seen in some cases of natural mitral insufficiency diagnosed by the surgeon palpating a regurgitant jet in the auricle; however, it has also been seen when no insufficiency was present and therefore may only be used to raise the suspicion of insufficiency. It is interesting also to note that the gradient in this patient has been reduced but the reduction is mainly by an elevation of end diastolic pressure in the ventricle.

DISCUSSION

Partial obstruction to blood flow at any point is marked by a differential in pressure on the two sides of the obstruction. This pressure differential will vary with flow, particularly with turbulent flow. By measuring the pressures on both sides of the mitral valve, we have one index of the degree of obstruction that may be present. With measurements before and after surgery, we obtain documentation of the improvement, assuming flow remained the same during both measurements. In the studies we have presented, it seems reasonable to assume that the flow is relatively unchanged, particularly if we use the aortic pressure pulse contours as an index of change in cardiac output. The cardiac rates have remained relatively constant.

In some of the patients in whom a gradient of pressure is still present despite the fact that by operation the orifice was enlarged to two finger widths, it is interesting to speculate as to the cause. In most such instances, the gradient has been reduced but not to a near zero level. We must, therefore, have some physiologic block still present, probably as a result of lack of pliability of the leaflets despite the fact that an adequate digital size has been achieved.

SUMMARY

Pressures in the left heart and aorta have been recorded simultaneously during operation. They show the physiologic processes of blood flow in these chambers. Observations before and after mitral commissurotomy have been presented.

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PLICATION OF THE ANNULUS IN THE CORRECTION OF MITRAL VALVULAR INSUFFICIENCY*

ELLIOTT S. HURWITT, PAUL W. HOFFERT, AND RUTHVEN FERREIRA**

The systolic sphincter-like action of the mitral annulus during closure of the valve in the hearts of living dogs has been clearly demonstrated in a motion picture from this laboratory, presented initially in the Surgical Forum in 1951.[1] Preliminary studies have shown that plication of the annulus in the region of the anterior commissure effectively corrects traumatic mitral insufficiency created in this location in dog hearts by means of a nerve hook, and that a surgical approach to the annulus from the epicardial surface of the left ventricle is feasible without compromising the myocardial blood supply.[2] The present report is concerned with efforts to create a more extensive experimental mitral insufficiency, and to develop techniques for the creation and correction of mitral insufficiency in the region of the posterior commissure.

METHOD

The nerve hook technique for creating mitral insufficiency, as previously described, often fails to yield adequate lesions. To produce insufficiency of greater magnitude, a rongeur has been substituted for the nerve hook Mongrel dogs were used, weighing from 35 to 60 pounds. All operations were performed under anesthesia maintained by a standard veterinarian solution of Nembutal administered intravenously. The left pleural cavity was entered by an anterolateral incision through the fourth interspace, with respirations maintained by a pump rhythmically delivering oxygen under positive pressure through an intratracheal tube The pericardium was incised vertically, anterior or posterior to the phrenic nerve, and the pericardial flaps held apart with traction sutures. A purse-string suture of No. 1 white braided silk on an atraumatic needle was passed around the base of the left auricular appendage, a non-crushing clamp was placed distal to this, [illegible] were [illegible] ienta- [illegible] ind a rongeur (Fig 1) was introduced into the auricular appendage and advanced until the tip was felt to lie just beyond the mitral annulus. The biting jaws of the rongeur were separated, and it was withdrawn slightly, so that a valve leaflet lay between the open jaws of the instrument At this point the biting jaws were closed, engaging and removing a portion of the leaflet, and the instrument was withdrawn from the auricular appendage Bleeding was controlled by tension [illegible] withdrawn, and a clamp placed on [illegible] felt either by re-inserting a finger [illegible]

* From the Surgical Research Laboratory and the Surgical Division, the Montefiore Hospital, New York City. This study was aided by a grant from the American Heart Association.

** [illegible]

of the left auricle. It has been possible, in this manner, to produce traumatic mitral insufficiency in the region of the anterior or posterior commissure, rotating the biting jaws of the rongeur to the appropriate position. The lesions in the region of the posterior commissure are of particular interest, since it is toward this location that attempts at the correction of human mitral insufficiency must be directed most frequently.

The technique of plication of the annulus is demonstrated in detail in Figure 2. A superficial elliptical incision is made in the epicardium at the site selected for plication. A superficial wedge of myocardium is excised within the limits of this incision. Three sets of imbricating sutures are then placed deeply, but not penetrating the ventricular cavity. These sutures are No. 0 braided silk, with an atraumatic non-cutting one-half circle needle at each end.* The needle at each end of the suture is passed from inside out of each margin of the defect. The superficial defect in the myocardium is converted to a deep wedge beneath the loops of the three sutures, employing a fine curved clamp as in a Ramstedt procedure. The gap is deepened for two-thirds to three-fourths of the estimated thickness of the wall

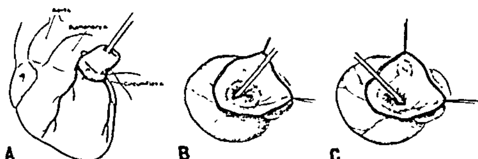


Fig 1. Mitral insufficiency created by introduction of rongeur into left auricle (*A*), directed at anterior commissure (*B*), and posterior commissure (*C*).

of the ventricle. The ends of each suture are tied together snugly, creating a pucker or pleat at the site of the defect

For plication in the region of the posterior commissure the operator stands at the subject's right side. The left forefinger is introduced into the left auricle, and acts as a guide to the exact location of the posterior regurgitant jet By gentle traction with the intracardiac finger, the heart may be rotated to the right, exposing the posterolateral aspect of the left ventricle and left auricle The region of the posterior commissure is identified; this is usually located deep to the angle present at the origin of the posterior descending coronary artery. As gentle pressure is made with the right forefinger over several points in this general area, the intracardiac finger will be able to determine which pressure point most completely eliminates the regurgitant jet[2] The plication procedure is carried out at this point exactly as anteriorly The sutures are tied by the first assistant, allowing the operator to keep his left forefinger in the heart so that the effect of the plication on the jet can be accurately evaluated. If the jet is not significanly diminish[illegible]

my [illegible]

the [illegible] rotate the heart adequately This should

* Supplied by the Ethicon Suture Laboratories, Inc., New Brunswick, N J.

be avoided whenever possible, since such marked dislocation of the heart may be tolerated poorly.

The base of the auricular appendage is secured by tying the purse-string suture, and the free margins are tied over a Gelfoam* pledget. The pericardium is loosely approximated. Pericostal sutures are placed around the

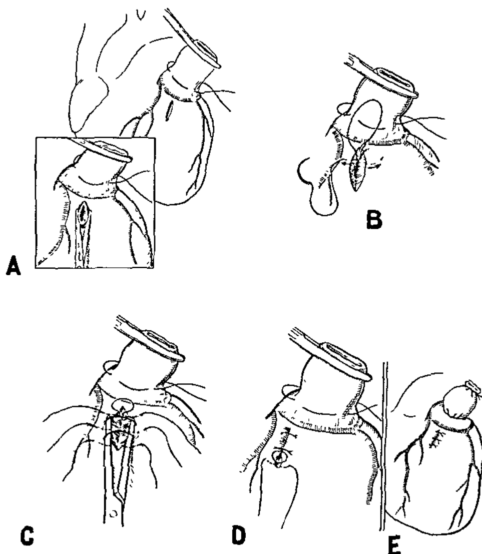


Fig. 2 Mitral insufficiency corrected by plication of annulus A, superficial myotomy, B, insertion of plicating suture, C, extension of myotomy, D and E, plication completed

ribs above and below the divided interspace, and the thoracic musculature and skin are repaired with continuous silk sutures.

RESULTS

As described elsewhere,[2] these procedures were evaluated by means of intracardiac and intravascular pressure curves with strain gauge manometers

* Supplied by the Upjohn Company, Kalamazoo, Michigan.

and electrocardiograms, simultaneously recorded.* These measurements were obtained prior to creating the lesion, after producing the insufficiency, and, when correction was attempted, after plication of the annulus.

Observations have been obtained on a total of 43 dogs, as of October 1, 1951. A detailed analysis of the results with the first 28 animals is contained in an earlier report.[2] Additional operations with the nerve hook laceration technique were performed on 8 dogs. These substantiated the initial impression, that plication of the annulus anteriorly could, in most instances, effectively correct the mild degrees of mitral insufficiency created in this area by this method (Fig. 3). In three of these dogs no attempt was made

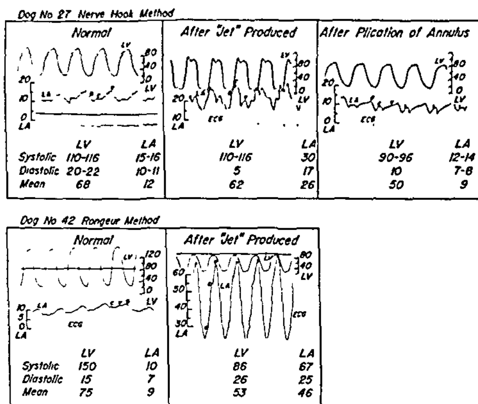


FIG. 3 Representative left auricular pressure curves with traumatic mitral insufficiency in region of anterior commissure Dog No. 27, nerve hook technique, with extensive C wave and pressure changes, improved by plication Dog No 42, rongeur technique, with massive insufficiency.

at immediate correction of the damage. These dogs were re-explored three to four months following creation of the lesion, and the pressure determinations were repeated In no instance was there any significant change from the curves obtained immediately following the creation of the lesion. Thus, there was no indication that the healing of the traumatized valve led to the creation of mitral stenosis.

The rongeur technique illustrated in Figure 1 has been attempted on 7 dogs to date, with 5 survivals, one fatality due to hemorrhage, and one cardiac death due to massive mitral insufficiency. By this method tissue is

* All the photographic tracings were taken with a multichannel electronic recorder, manufactured by Electronics for Medicine, Inc., New York City.

actually removed from the valve substance, with severance of adjacent chordae tendineae, resulting consistently in a significant regurgitation. This was corroborated at the time of sacrifice of 3 dogs, between the 57th and 77th postoperative days, with anterior rongeur defects, in whom no attempt at correction had been made. As with the nerve hook lesions, there was little change between the immediate and the late postoperative curves, indicating that spontaneous correction of the defects did not occur. Attempts at delayed plication in two of these animals, and at immediate plication in two others, proved to be ineffectual

An extreme example of the regurgitation that may result from this method is illustrated in Figure 3 Immediately upon production of the defect, great dilatation of the left auricle was observed, with a coarse palpable systolic thrill, followed by dilatation of the left ventricle The curves reflect the enormous "jet" The dog died 12 hours postoperatively, and autopsy disclosed destruction and absence of the entire anterior half of the septal (or "aortic") cusp, with disruption of the regional chordae Similarly placed but less extensive lesions were found in the other 4 dogs in this group, providing an anatomic explanation for the failure of plication to accomplish any correction.

The rongeur technique has been used in attempts to create traumatic mitral insufficiency in the region of the posterior commissure in 4 dogs Postmortem demonstration of inaccurately placed lesions correlates with pressure tracings that show little improvement after plication. A major effort at this time is being directed at more precise localization of the injury

DISCUSSION

This concept of anterior and posterior plication of the mitral valve annulus was derived from a study of the dynamics of valvular action, as documented by the motion picture. Dr. Denton Cooley has called our attention to the fact that Carrell, in 1910, referred to a "cuneiform resection of the anterior myocardium" as a possible technique for the treatment of mitral insufficiency Unfortunately, there are no details concerning the procedure, but he does mention that the technique was attempted in a dog, and that the dog's condition was good two months following the procedure [3] No other references to potentially similar methods have been found

It is probable that the technique of posterior plication will be applicable in human rheumatic mitral insufficiency more frequently than anterior plication. In this and most other surgical clinics, the regurgitant jet of mitral insufficiency has usually been located in the region of the posterior commissure This is well illustrated by the single human case of mitral insufficiency that we have attempted to correct by plication J.C, a 36 year old white male, underwent an exploratory cardiotomy at this institution on March 11, 1952 by Dr. Alan Bloomberg Mitral insufficiency was found, as indicated by a strong regurgitant jet on the palpating intracardiac finger, and by left auricular pressure tracings Postoperatively the patient's clinical condition had worsened considerably An attempt to correct the insufficiency by a plication procedure was made on July 20, 1954. The chest was entered in the usual manner, dense pleural adhesions were encountered, as well as adhesions between the pericardium and myocardium Pressure measurements showed no essential change from the left auricular curve of the previous operation.

A superficial vertical incision 1.5 cm. long was made in the epicardium extending from the region of the circumflex coronary artery, midway between the left anterior descending coronary artery and a large descending trunk located about 3 cm. to the left of the main left anterior descending artery. This incision was deepened superficially with a mosquito clamp, and three sets of deeply placed myocardial sutures were positioned and tied along each side of the incision. The myotomy incision was deepened with a curved clamp, so that the area to be plicated occupied a vertical length of 15 mm. and a depth in the heart muscle itself of 7 mm. The plication sutures were tied across this gap, and 2 deeply placed sutures tied to reinforce this Pressure measurements were then obtained from the left auricle and showed no significant change. The reason for this failure became obvious when the valve was explored with an intracardiac finger, and the insufficiency was noted to be in the region of the posterior commissure. During the plication procedure, the cardiac status was carefully monitored with a continuous electrocardiogram. At no time was there any indication that the manipulation associated with the plication procedure altered the status of the heart or was poorly tolerated. It became obvious, following this initial human experience, that a technique for posterior plication was required.

Rheumatic mitral insufficiency is not a standard lesion, and undoubtedly many of its variants will not lend themselves to correction by a single technique Unfavorable situations for the plication approach would include a rigidly calcified annulus, valve cusps held down tautly at the apex of a funnel by fused, contracted chordae tendineae; extensive myocardial damage; and a major loss of substance in the septal cusp The pressure curves in dog No. 42 (Fig. 3) illustrate this condition, as well as the danger of injury to this leaflet, with loss of its baffle function, during any intracardiac surgery. The difficulties of exposing the region of the posterior commissure in the normal dog heart may be considerably increased by the chamber enlargement and rotation of a human heart with rheumatic valvular damage. The increased thickness of left ventricular wall with long-standing mitral insufficiency requires that a myotomy of considerable depth must be performed Maximum benefit may be anticipated only if the sutures are tied sufficiently tightly to create a visible dip; this may be reinforced by one or two more deeply placed sutures.

It is possible that selected patients with mitral insufficiency may be helped by plication. In addition, occasionally, during a commissurotomy for rheumatic mitral stenosis, the fracture or division of the diseased tissue may extend too far into one of the commissures, resulting in a massive mitral insufficiency with rapid dilatation of the left auricle. Fatalities have been attributed to this complication, which has occurred at the anterior commissure more often than posteriorly Prompt application of the plication procedure may be life-saving under such circumstances.

SUMMARY

1 An experimental technique has been described for the production of mitral insufficiency in dogs, employing a rongeur to bite out portions of the mitral valve leaflets in the regions of the anterior and posterior commissures.

2 A technique for the plication of the annulus in these regions to correct these lesions is described

3. A case of human mitral insufficiency is reported in which correction by anterior plication was attempted

4. The applicability of posterior plication to some cases of human rheumatic mitral insufficiency, and of anterior or posterior plication to traumatic insufficiency accidentally produced during a commissurotomy for mitral stenosis is discussed.

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A STUDY OF PATENT DUCTUS TYPE SHUNTS IN THE DOG, USING A NEW TECHNIQUE FOR MEASURING GAS CONTENT OF THE MIXED PULMONARY ARTERY BLOOD*

DONALD J. FERGUSON, FLETCHER A MILLER, AND RICHARD L. VARCO

Application of the Fick principle to measurements of pulmonary blood flow in the presence of a systemic arterial shunt into the pulmonary artery is inaccurate. It is necessary to assume, for the purpose of calculation, that gas content of blood drawn from the pulmonary artery distal to the entrance of the shunt represents an equilibrium of mixed venous and systemic arterial blood, but, in fact, adequate mixing is not likely to occur.

In connection with a study of the relation between pulmonary artery blood pressure and flow, and the development of histologic changes in the vessels,[1, 2] it became necessary to devise a more satisfactory method of measuring the pulmonary blood gas content in the presence of a shunt This paper describes the method used, and some of the results obtained with it in dogs.

METHOD

Studies were made on 4 intact dogs and on 16 animals in which a shunt was made, and parts of the lungs were removed, as illustrated in Figure 1 In order to obtain a measurement of the mean gas content in the mixture of blood perfusing the lungs, the principle of using the lung as an aerotonometer, while rebreathing into a bag, was employed[3, 4] If the total lung area is so used, it is necessary to obtain equilibrium between the pulmonary artery blood and the respired gas in less than the time of one circulation, which is very short in the presence of a shunt, but if only part of the lung area is used for rebreathing, and if the remaining lung is adequate to support respiration, then the time for equilibrium is not limited. Gas may be re-

* From the Department of Surgery, University of Minnesota Medical School, and the Veterans Administration Hospital, Minneapolis

breathed as long as necessary, to reach constant composition, and can then be analyzed, and from the values for oxygen and carbon dioxide concentration so obtained, the corresponding gas content of pulmonary artery blood can be calculated. *This calculation involves an evaluation of interrelated* factors, including the effects of CO_2 content on the O_2 absorption curve, and

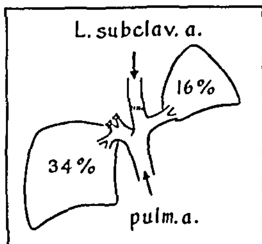


Fig 1 Diagram of operative result in dogs with shunt. Percentages of remaining lung tissue are based on averages from intact dogs (ref 2).

the hemoglobin content. To avoid the possible errors involved in such calculations, the equilibrated gas can be collected *in a tonometer and a small* sample of blood from the animal being studied can then be added and brought into equilibrium. The blood is analyzed by the usual Van Slyke

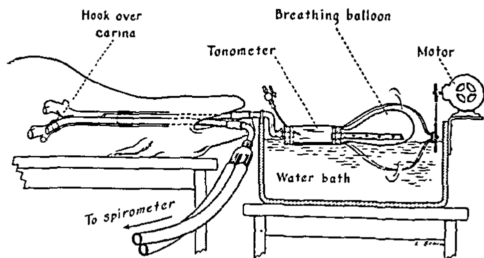


Fig 2 Apparatus for equilibrating a blood sample with rebreathed gas.

technique for oxygen or CO_2 content.[4] This method requires a large volume of gas in relation to the size of the blood sample, and introduces possible errors in collecting and manipulating the samples of blood and gas.

To avoid these difficulties, the method here described utilizes the apparatus shown in Figure 2. The bronchus of one lobe or one lung is con-

nected separately to a rebreathing bag, through a small tonometer which contains 6 ml of the animal's blood and is rotated in a water bath at 37° C. The capacity of the apparatus is adjusted to a volume a little greater than the tidal volume of the lung that is rebreathing. The remaining lung is connected separately for measurement of oxygen consumption and for alternate rebreathing The tracheobronchial tube with double lumen and 2 cuffs is shown in Figure 2. Calculations of cardiac output and shunt flow were made by conventional applications of the Fick principle Histologic technique and the evaluation of arteriolar lesions follows the description given elsewhere[1,2]

RESULTS

In 4 intact dogs, anesthetized with pentobarbital, the apparatus was connected as shown in Figure 2 A cardiac catheter was placed in the main pulmonary artery, and a cannula in the femoral artery A cuvette oximeter was used to evaluate the time required for equilibrium between pulmonary artery blood obtained from the catheter and the blood sample in the tonometer The latter was obtained from the femoral artery or vein. When the blood sample had initially an oxygen content lower than the final

Table 1. Comparison of Pulmonary Artery Sample with Tonometer Sample in Normal Dogs

			O_2 CONT., VOL. %		CO_2 CONT., VOL. %	
DOG		TIME OF EQUIL.	PA	TONOM	PA	TONOM
1	O_2 Left	23 min	7 89	8 24	—	—
2	O_2 Left	40 min	14 70	14 70	—	—
	O_2 Right	40 min	18 65	18 46	—	—
3	O_2 Left	10 min	19 02	18 90	50 95	48 00
4	O_2 Right	15 min	15 15	15 49	44 73	43 30

equilibrium value, the time for equilibrium was shorter than when the sample was initially too high in oxygen content. In the former case, 10 to 15 minutes was usually sufficient. When the oximeter showed equilibrium, samples were analyzed on the Van Slyke apparatus, with results as shown in Table 1

In 6 dogs with shunts, comparison was made between the values for shunt size obtained with the method here described, and those obtained by opening the chest and aspirating samples from 3 or 4 branches of the pulmonary artery into the same syringe (Samples were simultaneously obtained from the aorta and the right ventricle) The lung from which samples were drawn was the same as that used for rebreathing in the equilibrium tests. These data are shown in Table 2 At least two measurements of each type were obtained on each animal, which were within 15 per cent of their mean These two or more values were averaged to get the figures in the table Neither method is an accurate check on the other, since they were not carried out simultaneously, and both the thoracotomy and the rebreathing technique are very likely to alter pulmonary flow. Agreement between the two sets of observations therefore would not be expected to be any closer than it is, and the fact that they are in the same range of magnitude is all that is significant.

Of 16 dogs with a patent ductus type shunt, there were four which developed medial hypertrophy of the pulmonary arterioles after a period of about 5 months. These four had the largest shunts, as measured by the method here described; they averaged 66 per cent of left ventricular output, as compared with 48 per cent in the remaining dogs. The integrated mean pulmonary artery pressure in dogs with lesions averaged 28 mm. Hg as compared with 25 mm. Hg in those without lesions. A far more significant correlation of increased pulmonary artery pressure with the early development of severe medial and intimal lesions in pulmonary arterioles has been observed in animals with an end to end systemic–pulmonary artery shunt.[2]

Table 2. Comparison of Shunt Flow, Calculated as Percentage of Left Ventricular Output, by the Equilibrium Method, as Compared with the Method of Obtaining Blood Samples at Thoracotomy, in 6 Dogs

	SHUNT, % OF L.V. OUTPUT	
DOG	THORACOTOMY	EQUILIBRIUM
1	15	39
2	35	34
3	40	45
4	56	44
5*	65	62
6*	54	59

* Medial hypertrophy of pulmonary arterioles observed 5 months after operation. In the other dogs arterioles were normal after 12 to 18 months.

COMMENT

There are several errors inherent in the method described. Perhaps the most important is the assumption that the shunt flow is distributed between the two lungs in proportion to their size. In the present experiments, the data for Table 2 were obtained by right-sided rebreathing and right-sided thoracotomy. If the right-sided rebreathing was compared with left-sided rebreathing, agreement was only occasionally obtained, and in other animals consistent differences were observed with repeated measurements, suggesting that the shunt flow was directed more to one lung than to the other. In such cases the bilateral measurements would be necessary to calculate the shunt more accurately.

Another difficulty is the probable alteration in differential flow to the two lungs caused by rebreathing in one lung.[5] The magnitude of this change can be measured by alternate rebreathing on opposite lungs, in the absence of a shunt, and in four intact dogs so tested there was occasionally a large decrease of flow in the rebreathing lung.

SUMMARY

A method is described for determining the gas content of the mixed pulmonary artery blood entering one lung or part of one lung in the presence of a patent ductus type shunt. The method has been tested in intact dogs, and in dogs with shunts. Errors inherent in the procedure are discussed. Of 16 shunt dogs, the four having the highest flow through the shunt had pulmonary arteriolar medial hypertrophy after 5 months.

nected separately to a rebreathing bag, through a small tonometer which contains 6 ml of the animal's blood and is rotated in a water bath at 37° C. The capacity of the apparatus is adjusted to a volume a little greater than the tidal volume of the lung that is rebreathing. The remaining lung is connected separately for measurement of oxygen consumption and for alternate rebreathing. The tracheobronchial tube with double lumen and 2 cuffs is shown in Figure 2. Calculations of cardiac output and shunt flow were made by conventional applications of the Fick principle. Histologic technique and the evaluation of arteriolar lesions follows the description given elsewhere.[1, 2]

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DOG		TIME OF EQUIL.	PA	TONOM.	PA	TONOM.
1	O_2 Left	23 min	7.89	8.21	—	—
2	O_2 Left	40 min	11.70	11.70	—	—
	O_2 Right	40 min	18.65	18.46	—	—
3	O_2 Left	10 min	19.02	18.90	50.95	48.00
4	O_2 Right	15 min	15.15	15.49	41.73	43.30

equilibrium value, the time for equilibrium was shorter than when the sample was initially too high in oxygen content. In the former case, 10 to 15 minutes was usually sufficient. When the oximeter showed equilibrium, samples were analyzed on the Van Slyke apparatus, with results as shown in Table 1.

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trial septum around the lateral, inferior, and superior margins, leaving an intact base medially and the coronary sinus undamaged inferiorly. An additional incision is made in this flap of septum starting at the center of the periphery and extending toward the center of the base. This creates a bilobed flap of interatrial septum (Fig. 1). Each wing of the flap is rotated away from the central incision to cover the orifices of the venae cavae. The periphery of each wing of the flap is sutured to the atrial wall around

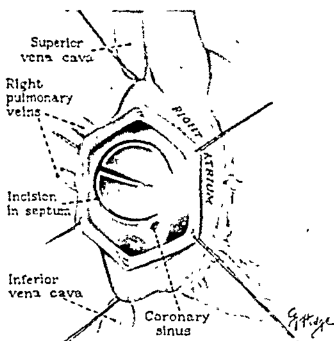


Fig. 1 View from right atrium.

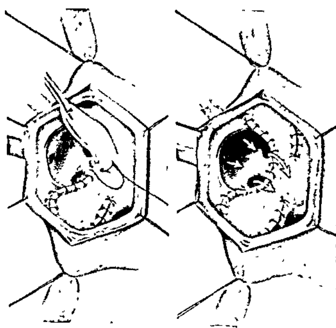


Fig. 2. See text.

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SURGICAL CORRECTION OF TRANSPOSITION OF THE GREAT VESSELS*

HAROLD M. ALBERT

So far there is no satisfactory treatment for the congenital cardiac anomaly of transposition of the great vessels. Transposing the pulmonary artery and aorta into their normal positions is not a satisfactory procedure because the coronary arteries would continue to receive venous blood. If, however, the systemic and pulmonary venous returns were transposed, the lesion, in effect, would be corrected An operation was devised in which an incision in the interatrial septum is made to produce flaps and by shifting and resuture of these flaps in new positions, transposition of the venous returns can effectively be done

PROCEDURE

After working out a rough technique using fresh dead hearts, twenty mongrel dogs, weighing between four and six kilograms, were used as the experimental animals

The animal is anesthetized with intravenous pentobarbital sodium, intubated so that artificial respiration can be maintained, then cooled in ice water to about 29° C. Further cooling to about 27° C occurs during the operation. The right pleural cavity is opened through the fourth interspace, dividing the fourth and fifth ribs at their angles. The azygos vein is ligated and the superior and inferior venae cavae have loose ligatures placed about them for later occlusion and release. The pericardium is opened widely to expose the right atrium, which is opened bloodlessly by using a Potts spoon clamp to pick up the atrial wall. The incision into the atrium is parallel to the venae cavae just anterior to the pulmonary veins and will be held open by traction sutures about its margin. The ligatures on the venae cavae are tightened and the heart allowed to empty before removing the clamp to enter the atrium. A C-shaped incision is then made in the intera-

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closed with the spoon clamp so that circulation can be restored and the atrial wall sutured without haste.

With this shifting of the interatrial septum in two planes, blood from the venae cavae and coronary sinus flows behind the two septal flaps into the narrowed left atrium, and thence into the left ventricle. Blood returning from the pulmonary veins empties directly into the right atrium and flows through the tricuspid valve into the right ventricle (Figs. 2 and 3). Thus a complete transposition of the venous returns can be done with a single suture line in from five to six minutes of arrested circulation. If the septum is completely excised and a plastic graft substituted the operation requires one additional minute (Fig. 4). With hypothermia these periods are within the allowable limits of time before anoxia causes brain damage.

RESULTS

Four animals died with ventricular fibrillation from air embolism on restoring circulation. One died with ventricular fibrillation before beginning the procedure, presumably because his temperature was allowed to go below 25° C The remaining 15 dogs survived from 22 minutes to four weeks before dying or being sacrificed. Autopsy showed the reason any animal survived such an abnormal circulation was that the most inferior of the left pulmonary veins was missed (not shunted) and there were occasional 1 to 3 mm defects between the sutures so that the vena cava blood was only 90 to 95 per cent diverted. Stenosis of the pulmonary veins was not found but three animals showed an estimated 10 to 20 per cent stenosis of the inferior vena cava

Two animals survived long enough (2 weeks and 4 weeks) to show healing was good, without thrombi, and without significant stenosis, but with effective transposition of the venous returns.

CONCLUSIONS

The experimental animal survives the intracardiac procedure but obviously should not survive the abnormal result, while the human with transposition of the great arteries may, by this operation, have as a result a normal circulation

the lateral, anterior, and medial margins of the vena caval orifices, the inferior flap is sutured between the vena caval opening and the atrioventricular valve and to the atrial wall immediately surrounding the caval orifice The straight sides of the flaps, resulting from the central incision, are sutured to the posterior wall of the left atrium around the openings of the pulmonary veins (Fig. 2). As the last stitch is placed, the chest is filled with Ringer's solution and the inferior vena cava is released When air ceases to bubble from the heart, the suture is tied and cut and the heart

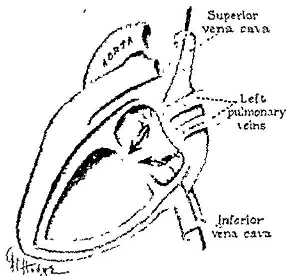


Fig 3 View from left atrium after completion of the shunt.



Fig. 4 View from right atrium with plastic graft in place

artery) were isolated, and all were ligated and cut except the internal mammary. The main continuation of the subclavian artery was securely ligated but not cut. The internal mammary artery was dissected free of all branches from its origin to its site of entrance into the myocardium. Heparin was then administered intravenously. It was possible to place a special T-shaped cannula into the subclavian artery (Fig. 1). The upper limb of the T was connected to the reservoir, and this, in turn, to a constant pressure tank. To fill this reservoir, blood was allowed to enter from the proximal side of the subclavian and flow up into it. A bulldog clamp was applied on the subclavian artery proximal to the cannula, and then perfusion was performed at any desired constant perfusion pressure.

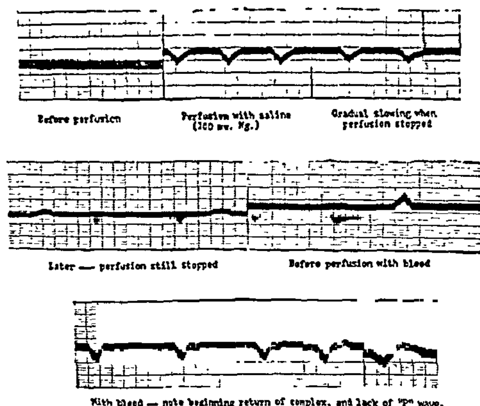


Fig. 2. Electrocardiogram, note the gradual recovery of an integrated beat following perfusion through the implanted internal mammary artery with oxygenated blood

In order that the blood pressure be controllable, a constant pressure reservoir was attached to the animal's femoral artery. In the event of a drop in blood pressure, blood flowed in from the reservoir, or, with a rise, it would flow back into the reservoir.

Recordings were obtained of the blood pressure and pulse, and electrocardiograms were taken throughout the course of the experiments.

In addition, experiments were performed on animals whose hearts had been placed in arrest owing to exsanguination. The flow results were compared with those obtained prior to death in the same animal. Note was made of any return of fibrillation or of localized heartbeat that had been completely still for as long as 30 minutes. This was recorded either by the Cushney Myocardiograph[3] or by the electrocardiograph (see Figure 2).

A STUDY OF THE AMOUNT OF BLOOD AND OXYGEN DELIVERED TO THE MYOCARDIUM THROUGH THE IMPLANTED MAMMARY ARTERY*

WILLIAM KEITH BULLER AND ARTHUR M. VINEBERG**

In previous publications it has been shown that the internal mammary artery, after implantation into the left ventricular myocardium, sends out branches which anastomose with the arterioles of the myocardium. The quantity of blood supplied to the myocardium by the implanted internal mammary artery has been measured in a series of 8 animals which had survived ligation of the anterior descending branch of the left coronary. The blood flow studies through the internal mammary were performed at intervals of five to seven months after implantation.

METHOD

Under general anesthesia, using chloralose and urethane, the left chest was opened widely from the fifth rib up to and including the first rib. The left subclavian artery and its branches (including the internal mammary

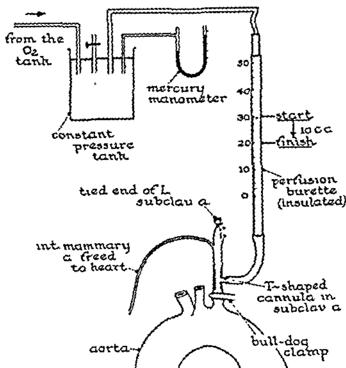


Fig. 1 Flow apparatus, diagrammatic representation of the perfusion system used in these experiments.

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** This work was carried out in close collaboration with F. C. MacIntosh, Ph.D., F.R.S., and Peter Oborin, M.D., M.Sc., in the Department of Physiology, McGill University, Montreal

of the artery was closed and the sixth intercostal alone was opened, the latter delivered 45 cc. per minute.

It has been demonstrated in each experiment that the perfused blood flow through the implant is directly proportional to the perfusion pressure when other factors remain constant. The two seemed to vary as a straight line function. When they were plotted on a graph, it was possible by extrapolation to arrive at the probable pressure at which return flow through the implant would begin. This finding was reduplicated experimentally, with return flows back through the implant as high as 1 cc. per minute being recorded when the perfusion pressure was reduced to zero.

The actual measured flow varied within wide limits, although for each implant the results were completely reproducible. Flows varied in the several experiments from 1 to 20 cc per minute when the blood pressure and the corrected perfusion pressure were approximately equal. Flows as high as 59 cc. per minute were possible in one instance with a large pressure differential.

Some of the experiments were affected by sludging and rouleux formation, and the flow results then become quite inaccurate. This process was recognized by the diminished flow recorded with successive perfusions at the same perfusion pressure, other factors remaining constant. Although the animals were thoroughly heparinized, the apparatus silicone coated, and the blood not left in the burette any longer than was necessary to take a reading, this complication occasionally occurred.

The introduction of epinephrine into the perfused blood was observed to increase the rate of flow temporarily. This is actually what one would expect if the implant has anastomosed with the coronary arterial system, for the effect of epinephrine on the coronary flow is to increase it. Interestingly enough, the major cause of increase in this case does not seem to be an increase in cardiac output as is commonly supposed, but a direct action on the vessels.

When the recently arrested heart was perfused through the implant in six out of eight flow experiments, a remarkable recurrence of myocardial activity was noted. If the heart had stopped beating in ventricular fibrillation, then a resurgence of the fibrillation became evident. If there was no fibrillation, the heart then often returned to activity with an organized beat, but localized to the left ventricle. This implies that the myocardium was not completely dead, and still retained the ability to absorb metabolites and oxygen from the blood reaching it through the internal mammary implant. Most important though, it implies that the blood being perfused through the implant was reaching the left coronary arterial tree and the myocardial fibers (see Fig. 2).

Further evidence of this was obtained in the one experiment in which oxygen saturation studies were performed during the postmortem perfusion. T[illegible] 23 mm. Hg), a [illegible] per minute. T[illegible] throughout the perfusion (several samples). The first coronary sinus samples were discarded, and the first recorded sample was 60 per cent. Successive samples gradually fell to 40 per cent level, at which point the heart was noted to start beating. Possibly the starved myocardial fibers were removing more and more oxygen from the blood when recovering.

In one instance, postmortem comparison of blood oxygen levels was performed between the perfused blood and that returning through the coronary sinus in the course of perfusion. The first specimens were discarded, and not until a definite flow had been re-established through the sinus was the oxygen level checked. A direct-reading colorimeter was used

The method of blood flow measurements is simple. Serious errors which might occur in more complex methods are readily detected using this technique The apparatus answers the main criteria, which are (1) simplicity, (2) reasonable accuracy, (3) ease of construction, (4) ease of application, and (5) ease of interpretation

Fresh blood was used for each experiment in order to minimize the activity of any vaso-active substances The flow was measured over the central range of the burette, and a calculation introduced to correct for



Fig 3



Fig 4

Fig 3 Plastic injection cast of the animal upon which the flow rates and oxygen studies are reported Note the internal mammary implant in white with its branching, and the white plastic solution in the anterior descending branch.

Fig 4 Plastic injection cast, dissected to show the anastomosis Note the white plastic injected through the internal mammary implant in the anterior descending branches of the left coronary

the weight of the blood column. Any tendency to sedimentation was circumvented simply by not allowing the blood to stand in the burette between readings. The burette was insulated, although no other attempt was made to maintain the blood at body temperature The timing of the flow was by means of a stopwatch, and was checked in each case by more than one person, and by more than one trial

Since a pulsatile force produces a greater flow through vessels than a constant perfusion pressure, the flow values obtained would, of necessity, be lower than those existing in the functioning artery The mean flow apparatus was sufficiently accurate to give an indication of the potentialities of the implant procedure.

RESULTS AND DISCUSSION

The total possible flow through an internal mammary artery was measured in situ in the chest. This was found to be 74 cc. per minute. When the end

It is interesting to note, too, that the coronary sinus is reputed to return some 60 per cent of the coronary venous blood normally. Measurement performed during this experiment revealed that 55 per cent of the blood perfused through the implant was recovered from the coronary sinus, and is further evidence of an anastomosis with the coronary arterial system.

Table 2. Flow Studies (Animal No. 33w)

BLOOD PRESSURE, MM	PERFUSION PRESSURE, MM	CORRECTED P.P., MM	COMPENSATOR PRESSURE, MM	TIME FOR 10 CC., SEC	FLOW, CC./MIN.
(1) 102	100	123	70	20 8	28.9
(2) 96	70	93	constant	29 8	20.5
(3) 92	40	63	constant	56.7	9.5
(4) 90	10	33	constant	243 0	2 5
(5) 88	Zero	23	constant	Reverse flow of 1 cc./min (at the mid burette level)	
(6) 86	100	123	constant	19 0	31.6
(7) 86	139	153	constant	14.1	42.8
(8) 82	160	183	constant	11.7	51.4
EFFECT OF LOWERING THE BLOOD PRESSURE.					
(9) 72	160	183	50	10 2	59 4
64	130	153	50	12 3	48 8
52	100	123	50	14.2	42.5
46	70	93	50	20 9	29.7
42	40	63	50	33 2	18 8
EFFECT OF BLOOD TRANSFUSION					
(10) 86	100	123	60	19.4	31.2
84	100	123	60	19.2	31.4
EFFECT OF EPINEPHRINE (500 MG.) INTRAVENOUSLY					
(11) 82	100	123	60	22 5	26 6
80	100	123	60	19.9	30 8
86	100	123	60	19 0	31 6
EFFECT OF EPINEPHRINE (500 MG) INTO INFUSED BLOOD (through implant)					
(12) 80	100	123	60	15.5	38.7
78	100	123	60	14.5	41.6
74	100	123	60	13 7	43.8
78	100	123	60	15 7	38.2
THE ANIMAL WAS THEN BLED TO DEATH INTO COMPENSATOR					
(13) Zero	100	123	Zero	11.2	54 0

Note Artery implanted 11 5 53, anterior descending branch ligated, 12/1/53, flow studies, 3/31/54.

EVIDENCE OBTAINED THAT THE IMPLANT ANASTOMOSES FUNCTIONALLY WITH THE CORONARY ARTERIAL SYSTEM

1. The flow through the implant is a direct function of the perfusion pressure.

2 The perfusion flow through the implant is directly related to the arterial pressure. When the pressure increases, the flow decreases. If the perfused blood was passing into the venous system directly, the effects of changes in arterial pressure would be much less obvious.

3. When epinephrine is introduced into the perfused blood, it has the same effect on the flow through the implant as it would on the coronary arterial circulation That is, epinephrine causes an increase in flow.

4. Reverse flow will occur through the implant when the perfusion pressure is low enough to produce a relatively large pressure differential between it and the blood pressure. A measurement of the pressure exerted

Table 1 A Collective Review of the Blood Flow Experiments

ANIMAL NO	INTERVAL 1 (Implant to ligation)	INTERVAL 2 (Ligation to flow study)	CAROTID B P (mm Hg)	CORRECTED P P (mm Hg)	FLOW (cc, min)	RETROGRADE FLOW	ANASTOMOSIS (Studied by injection)
1 (control)			60	102	45 cc (6th intercostal) in situ		
5	16 weeks		120	141	5.7 cc	0.4 cc at 39 mm	Yes
14w	9 weeks	11 weeks	40	72	11.2 cc		Yes
33w	4 weeks	16 weeks	66	93	20.5 cc	1.0 cc. at 23 mm	Yes
15w	4 weeks	15 weeks	100	137	6.9 cc	1.3 cc at zero	Yes
1w	8 weeks	11 weeks	POSTMORTEM FLOW 12.8 cc./min at 100 mm				Yes
128	8 weeks	20 weeks	POSTMORTEM FLOW 11.3 cc./min at 120 mm				Yes
111	8 weeks	12 weeks	90— 110—	120— —	1.5 cc 1.1	0.8 cc at 20 mm.	No (patent)
521	4 weeks	17 weeks	110	131	2.7 cc	present	No (patent)

Note The comparisons are made at the level at which the perfusion pressure (P P) approximates the mean blood pressure (B P)

EXPERIMENTAL EVALUATION OF EXTERNAL SHUNTS FOR BY-PASSING THE THORACIC AORTA*

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Although in the process of resection of aortic aneurysms the descending thoracic aorta has been obstructed for periods up to forty-seven minutes without producing heart failure or neurologic damage,[1] experimental and clinical evidence indicates that this maneuver is unsafe save in coarctation with its attendant expanded collateral circulation. Local refrigeration[2] and generalized hypothermia[3,5] have been shown to have some protective value in the prevention of hindquarter paralysis following aortic occlusion. Until measures to minimize the risk of ventricular fibrillation or cardiac standstill in adults under hypothermia are found,[6] endeavors to develop an adequate and safe means of diverting the blood flow by an external shunt during the period of aortic occlusion would appear worthwhile. Important advances in the solution of this problem have already been made by others,[7,8] but many technical and hemodynamic questions remain to be answered.

The authors have reported the successful clinical use of an external shunt in the removal of the first portion of the descending thoracic aorta.[9] The present report concerns hemodynamic studies intended to elucidate the optimum caliber and physical characteristics of the shunt they have described, and to report the results of 20 attempts in dogs to excise the aortic arch with graft replacement.

HEMODYNAMIC STUDIES

Method

Adult mongrel dogs were anesthetized with intravenous Nembutal, and 100 per cent oxygen under intermittent positive pressure was administered by means of a Bureau of Mines Neophore valve through an endotracheal tube. The descending aorta was mobilized through the bed of the excised left sixth rib. A U-shaped heterologous graft shunt 30 cm. in length was anastomosed with two end to side anastomoses spaced 5 cm. apart employing the Beck-Potts exclusion clamp. The graft was prepared by anastomosing selections from two heterologous aortas taken from 200 pound pigs.

Following establishment of the U shunt, the animal's lungs were ventilated with 100 per cent oxygen by means of a Benedict-Roth apparatus activated by a windshield wiper respirometer. A continuous record of oxygen consumption was thus obtained. Blood samples were obtained anaerobically in Mepesulfatized† syringes directly from the pulmonary artery and aorta

* From the Department of Surgery, Albany Medical College, Albany, New York, and Naval Medical Research Institute, National Naval Medical Center, Bethesda, Maryland. Also supported by a grant from the Albany County (New York) Heart Association. The opinions and conclusions expressed in this paper are those of the authors and are not to be construed as official or necessarily reflecting those of the Medical Department of the United States Navy, or of the Naval Service at large.

† Mepesulfate, Hoffmann-La Roche.

by this reverse flow indicates that the anastomosis is with small vessels, probably of the size of arterioles

5. The recurrence of cardiac pulsation or fibrillation when the recently stilled heart was perfused through the implant indicates that the myocardium can receive nutriment from this source

6 Finally, the ability of the myocardium to utilize oxygen provided through the implant indicates the functional value of the anastomosis

SUMMARY

The blood flow characteristics of the implanted internal mammary artery have been investigated by means of a constant pressure perfusion apparatus. Evidence was obtained from these studies that a functional anastomsis existed between the implant and the left coronary arterial system, and that the implant was capable of delivering oxygen to the myocardium The volume of flow was directly proportional to the extent of the anastomosis and to the perfusion pressure, and inversely proportional to the mean blood pressure The implanted internal mammary artery is capable of allowing very high flows of oxygenated blood to the myocardium

Table 3 Myocardial Consumption of Oxygen from Blood Supplied through the Implanted Internal Mammary Artery, 5 Months after Implantation

CAROTID PRESSURE, MM HG (DOG KILLED BY BLEEDING, HEART IN ARREST)	INTERNAL MAMMARY ARTERY PERFUSION PRESSURE MM HG	BLOOD FLOW THROUGH INT MAMMARY IMPLANT, CC MIN	CORONARY SINUS FLOW CC MIN	OXYGEN SATURATION: INT MAMMARY BLOOD, % SATURATION	OXYGEN SATURATION: CORONARY SINUS BLOOD % SATURATION	REMARKS
0	123	54	30	98	60	
0	123				55	
0	123			9	55	
0	123			98	42	
0	123			98	40	Heart started to beat

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shunt the arch was excised and replaced by a homologous freeze-dried, ethylene oxide vapor-sterilized graft in all but one instance, wherein a fresh graft was employed.

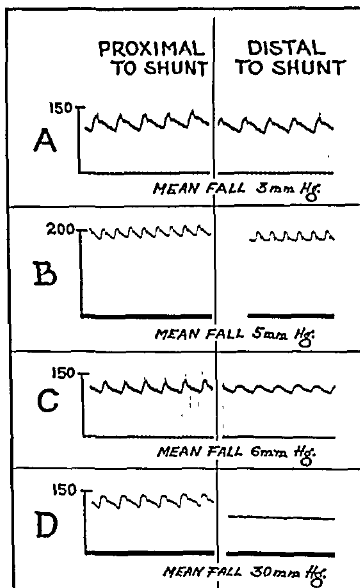


Fig 1. Representative pulse pressure recordings from aorta proximal and distal to shunts. *A*, Shunt closed, aorta patent. *B*, Heterologous shunt, aorta occluded. *C*, 10 mm. Tygon shunt, aorta occluded. *D*, 6 mm. Tygon shunt, aorta occluded.

Experience has shown that the procedure is best done in the following steps:

1. Mobilization of the arch.
2. Anastomosis of homologous aorta shunt sleeves to ascending and descending aorta.
3. The ascending and descending aortic limbs of the shunt are first put into operation and the "elbow" cannula inserted into the ascending aorta where it is tied in place by umbilical tape padded by the preserved aortic fat pad. This is a very important step. If, instead, the brachiocephalic artery

for the determination of cardiac output Determination of oxygen content of the blood was performed after the method of Van Slyke and Neill. Blood pressure was recorded directly from the aorta through a 1.5 cm. 25 gauge needle by means of a Hathaway blood pressure amplifier and oscillograph.

With the U shunt clamped off and the aorta patent, blood pressure was recorded from the aorta just above and just below the shunt. Cardiac output was determined and the shunt was then opened and the aorta clamped off. After a period of two minutes, cardiac output and blood pressure measurements were repeated. In sequence identical measurements were made with hog aorta and Tygon shunts of 10 mm. and 6 mm. internal diameter. The average shunt length was 30 cm In five animals all three shunts were studied. In four additional animals studies were limited to 10 mm. and 6 mm. Tygon shunts.

Results

None of the three shunts investigated produced any significant change in cardiac output, although small random variations were noted. The pig aorta shunt produced only a small rise in mean aortic pressure on the cardiac side, averaging 3 mm Hg in mean pressure. Pulse pressure distal to the shunt was well maintained and the average drop in mean pressure was 6 mm. Hg. Studies on the larger Tygon shunt (10 mm internal diameter) revealed a similar effect on aortic pressure on the cardiac side, the average increase in mean pressure being 5 mm. Hg. In most instances pulse pressure was well maintained in the distal segment, although in one experiment it was reduced to 12 mm. Hg. The average fall in mean pressure was 10 mm. Hg. Experiments with smaller Tygon shunt (6 mm internal diameter) showed a much greater effect on aortic pressure proximal to the by-pass. The average rise in mean pressure was 11 mm. Hg. In every case the pulse pressure was almost completely abolished distally, and the average fall in mean pressure through the shunt was 15 mm of mercury (Fig. 1).

Cardiac work, estimated as the product of mean pressure and cardiac output, was not significantly altered in these studies. Failure to demonstrate an increase in cardiac work with the 6 mm. shunt in operation is believed to result from hypotension which almost uniformly attended its use

Mean blood flow through the 6 mm shunt was 1100 ml. per minute. The very small pressure drop noted with the 10 mm. and heterologous shunts precluded calibration of these tubes as a flow meter. However, failure to produce a significant pressure drop necessarily indicates only slight reduction in aortic blood flow.

EXPERIMENTAL AORTIC ARCH RESECTION AND RECONSTRUCTION BY GRAFT

Method

Adult mongrel dogs were anesthetized with intravenous Nembutal, and 100 per cent oxygen under intermittent positive pressure was administered through an endotracheal tube. The aortic arch and branches were mobilized through the bed of the excised left fourth rib with the animal in the lateral position. The aortic arch was by-passed by a three-limbed shunt connecting the ascending aorta, brachiocephalic artery and descending aorta, in the manner depicted in Figure 2. With the blood diverted through the

ing 8 are as follows: 6½ weeks—aneurysm and rupture of the graft; 7 days—wound infection and empyema; 18 hours—thrombosis of fresh homologous graft (only fresh graft employed in series), two died early postoperatively and 6 hours, respectively, of pulmonary edema; 12 hours—unknown cause, though transfusion reaction was suspected, and two died at 3 and 4 days, respectively, of rupture of the graft through the impression left by a Potts serrafin clamp used to facilitate the brachiocephalic anastomosis.

The dog which died of aneurysm of the graft with rupture is of unusual interest. This homologous freeze-dried, ethylene oxide-sterilized graft came from the same batch as that employed in our sole long-term survivor and was prepared according to the method of Pate and Sawyer[11] with exposure to ethylene oxide vapor for two hours. It would appear that the increased pressure and turbulence factors to which an aortic arch and side branches graft is subjected may prove to be a more valid test of graft preparations than abdominal aortic grafts where the flow is more nearly linear.

The animal which developed a wound infection and empyema differed from the rest in that the left subclavian artery was not anastomosed to the graft. It was evident early postoperatively that the circulation to the upper margin of the thoracotomy wound was thereby impaired and set the stage for a wound infection.

None of the animals who survived aortic arch resection and grafting evidenced any demonstrable neurologic deficit.

Comment

Although only slight hemodynamic advantage was demonstrated in the performance of the heterologous graft shunt as compared with the large-bore Tygon-siliconed-glass shunt, the vascular shunt offers other advantages. These are: (*a*) a continuous smooth antithrombogenic intimal lining, (*b*) the elasticity of the graft permits excellent mobility of the shunt even while in operation, thereby facilitating the surgery for which the shunt is being employed, and (*c*) the material is readily available and can be stock-piled in the sterile freeze-dried state.

In the preparation of three-limbed heterologous shunts, advantage has been taken of the fact that the cow's aorta has but one branch. With care this can be removed from the cow with attached carotid and subclavian arteries of adequate length for a shunt. With the stump of the brachiocephalic truncus oversewn, converting it into a manifold, and undesirable side-branches ligated, an excellent shunt of large caliber and free of joints can be fashioned. This shunt is now under investigation and appears promising.

An attempt to evaluate the desirability of the adjunct use of hypothermia or hypotension in aortic arch resection employing heterologous graft shunts is planned.

Conclusions

1. A surgically feasible method for shunting blood about segments of the thoracic aorta is presented

2 Experimental data demonstrate that properly selected shunts satisfactorily sustain cardiocirculatory dynamics; cardiac output and cardiac work are not significantly altered, mean pressure, pulse pressure, and adequate blood flow distal to the shunt are preserved.

is occluded in an effort to cannulate it with the third limb of the shunt prior to tying the ascending aortic elbow cannula in place, the resulting carotid reflex rise in blood pressure will almost uniformly disrupt the ascending aortic sleeve suture line. (The first portion of the ascending aorta in dogs is notoriously friable[10].)

4. The brachiocephalic artery is then cannulated with the third limb of the shunt, relying momentarily upon the vertebral branch of the left subclavian artery for circulation to the brain

5. With all three limbs of the shunt in operation, the left subclavian artery is occluded, the arch resected, and the graft sutured in place. The

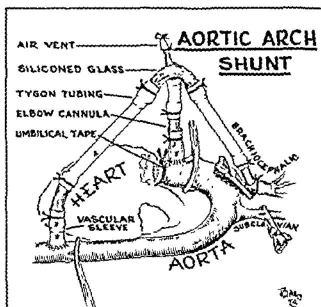


Fig 2. Showing three-limbed Tygon and siliconed-glass shunt in place with total by-pass of the aortic arch

anastomoses are effected in the following sequence. proximal aorta, distal aorta, brachiocephalic and left subclavian

6. The shunt limbs are then removed in the reverse order of their insertion, and the defects in the aorta and brachiocephalic are repaired

Results

Aortic arch resection and reconstruction by grafts was attempted in 20 dogs. The operative procedure was technically achieved in 9. In the 11 unsuccessful attempts, 5 died as a result of disruption of the proximal sleeve suture line because of reflex hypertension induced by occlusion of the brachiocephalic artery. This experience led to the introduction of the "elbow" cannula tied into the ascending aorta prior to cannulation of the brachiocephalic artery as indicated in step 3 of the operative procedure. Four others died of technical failure arising after the shunt was satisfactorily in operation.

Of the 9 dogs who survived the operation, one is living and clinically well 10½ months postoperatively, with good bilateral carotid, brachial and femoral pulses. The duration of survival and cause of death in the remain-

shock.[4] The experiments reported in this paper were set up to determine if the liver, intestines, or kidneys are the primary organs injured or if death is caused by a mechanism resembling tourniquet shock in which a large area of vascular bed is rendered ischemic.

METHOD

Healthy mongrel dogs weighing between 6 and 15 kg. were used in these experiments. After fasting for 12 hours, they were anesthetized with Nembutal, 30 mg. per kilogram, given intravenously. Using aseptic technique, the left chest was entered through the eleventh intercostal space while the

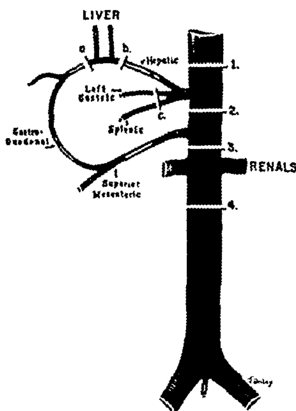


Fig 1 Position of clamps in various experiments.

lungs were mechanically inflated by intermittent positive pressure. The pleura was dissected from the lower thoracic aorta and the origin of the celiac and superior mesenteric arteries were cleared of fat.

In order to evaluate the effect of temporary aortic occlusion on various organ systems below the diaphragm the following sites of clamping were selected.

Experiment A. Above the celiac artery (Fig. 1, *1*).

Experiment B. Below the celiac artery (Fig. 1, *2*) while temporarily occluding the left gastric and splenic branches of the celiac artery (*c*) with a rubber clamp for the exact period of aortic occlusion. Prior to the period of clamping, a small incision was made through the diaphragm and through the gastrohepatic ligament. The gastroduodenal artery was ligated perma-

3. It is the opinion of the authors that a heterologous vascular shunt offers the most satisfactory solution to the problem.

4. One dog has survived 10½ months following homologous replacement of the aortic arch

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THE MECHANISM OF DEATH FROM THORACIC AORTIC OCCLUSION*

W. STERLING EDWARDS, OWEN K. TIDWELL, AND CARLOS R. LOMBARDO

Aneurysms of the thoracic aorta, especially those of the descending aorta distal to the left subclavian artery, are now amenable to surgery in many instances It is possible in saccular aneurysms to perform a lateral excision of the dilatation without interrupting blood flow.[1] Fusiform dilatations can be resected and the defect bridged with an aortic graft.[5] Nevertheless, resection and grafting of the thoracic aorta where there has been no previous stimulus to collateral artery dilatation is a dangerous procedure. Two frequent complications occur in animal and man after prolonged interruption of the thoracic aorta: (*a*) paraplegia from spinal cord anoxia, and (*b*) death from severe hypotension after release of occlusion.[2, 3] In the dog, prolonged aortic occlusion just above the diaphragm causes no paralysis, but a large percentage of animals die shortly after releasing the occlusion from a condition clinically and pathologically resembling irreversible hemorrhagic

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shock.[4] The experiments reported in this paper were set up to determine if the liver, intestines, or kidneys are the primary organs injured or if death is caused by a mechanism resembling tourniquet shock in which a large area of vascular bed is rendered ischemic.

METHOD

Healthy mongrel dogs weighing between 6 and 15 kg. were used in these experiments. After fasting for 12 hours, they were anesthetized with Nembutal, 30 mg. per kilogram, given intravenously. Using aseptic technique, the left chest was entered through the eleventh intercostal space while the

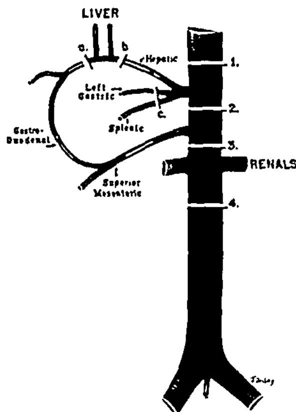


Fig. 1. Position of clamps in various experiments

lungs were mechanically inflated by intermittent positive pressure The pleura was dissected from the lower thoracic aorta and the origin of the celiac and superior mesenteric arteries were cleared of fat.

In order to evaluate the effect of temporary aortic occlusion on various organ systems below the diaphragm the following sites of clamping were selected·

Experiment A Above the celiac artery (Fig. 1, *1*).

Experiment B. Below the celiac artery (Fig. 1, 2) while temporarily occluding the left gastric and splenic branches of the celiac artery (*c*) with a rubber clamp for the exact period of aortic occlusion. Prior to the period of clamping, a small incision was made through the diaphragm and through the gastrohepatic ligament. The gastroduodenal artery was ligated perma-

nently immediately beyond the last branch of the hepatic artery to the liver (Fig. 1, *a*). This experiment was designed to study the effect of occlusion of the lower thoracic aorta with interruption of flow to the gastro-intestinal and genitourinary tracts, while maintaining arterial flow to the liver.

Experiment C. Below the superior mesenteric artery (Fig. 1, 3) ligating permanently the gastroduodenal artery at point *a* as in experiment B, and temporarily occluding the hepatic artery (Fig. 1, *b*) for the exact period of aortic occlusion This experiment was designed to study the effect of occlusion of the aorta plus the entire arterial supply to the liver while maintaining the blood supply to the spleen and gastro-intestinal tract. A careful search was made for phrenic arterial branches arising from the aorta, since these have been shown to provide collateral arterial supply to the liver,[7] and all such branches were ligated

Experiment D Below the superior mesenteric with both the celiac and superior mesenteric arteries unoccluded (Fig. 1, 3).

Experiment E Below the renal arteries. In this small series of animals the aorta was exposed through a mid-line upper abdominal incision (Fig. 1, 4).

At the end of the planned period of occlusion, clamps were released, the diaphragm and thoracic incisions were carefully closed after reinflation of the lungs No penicillin or other antibiotics were given to these animals post-operatively. They were observed for 48 hours, and those living at the end of this period were counted as survivors. Most of the deaths occurred within twelve hours after surgery, and resembled death from irreversible shock in that bloody diarrhea was an almost constant sign shortly before demise.

To investigate further the possibility that intestinal ischemia is lethal because of absorption of bacteria or their toxins through the intestinal wall, the following experiments were carried out. Using sterile technique the superior mesenteric and celiac arteries were dissected out at their origin from the aorta, through a lower thoracic incision. A number of animals were studied as a control series by clamping these two vessels simultaneously for periods ranging from 1 hour to 2 hours and 10 minutes. A second experimental series was carried out in dogs prepared by pretreatment with Achromycin* (tetracycline) in oral doses of 2.5 grams twice a day for three days, and a priming dose of 5 0 grams ingested 6 hours before occlusion. A third group of animals was pretreated with procaine penicillin G, 600,000 units intramuscularly daily for two days and a priming dose of 600,000 units one hour before occlusion. The mortality rates were compared after release of clamping in these three groups.

RESULTS

The number of animals in each group and the mortality for each period of aortic occlusion is summarized in Table 1. Occlusion above the celiac artery was not well tolerated even for 1 hour, the mortality was 80 per cent Occlusion for 1 hour and 30 minutes resulted in death in 100 per cent. In experiment B, where the entire gastro-intestinal tract was ischemic but arterial supply to the liver maintained, there was 60 per cent mortality after 1 hour and 30 minutes, and 80 per cent after 2 hours. If the hepatic arterial supply was occluded along with the aorta but the superior mesenteric artery remained open (experiment C), there was only 10 per cent mortality after 2

* Achromycin was supplied by the kindness of Lederle Laboratories, through Dr. Dale Archer.

hours. Three hours of occlusion above the renals caused death in 10 per cent in the first 48 hours from a shocklike state, but a majority of the surviving animals died in four to ten days from severe renal damage. Occlusion below the renals is tolerated for long periods of time as indicated by survival of all animals with occlusion for 5 hours.

Table 2 summarizes the results of simultaneous occlusion of the superior mesenteric and celiac arteries for various periods with and without antibiotic premedication. The principal findings of this experiment were: (*a*) the gradual rise in mortality in the untreated animals as occlusion was prolonged, (*b*) the significantly lower mortality for similar periods of occlusion in the dogs treated with Achromycin and penicillin as compared with controls, and

Table 1. Results of Aortic Occlusion at Various Levels

		POSITION OF CLAMPS				
TIME		EXPT A ABOVE CELIAC	EXPT B ABOVE SUPERIOR MESENTERIC HEPATIC ARTERY OPEN	EXPT C BELOW SUPERIOR MESENTERIC ALL HEPATIC ARTERIES OCCLUDED	EXPT D ABOVE RENALS	EXPT E BELOW RENALS
1 Hour	Number	10				
	Deaths	8				
	Mortality	80%				
1 Hour 30 Minutes	Number	5	10			
	Deaths	5	6			
	Mortality	100%	60%			
2 Hours	Number		10	10		
	Deaths		8	1		
	Mortality		80%	10%		
2 Hours 30 Minutes	Number			5		
	Deaths			5		
	Mortality			100%		
3 Hours	Number				10	
	Deaths				1	
	Mortality				10%	
5 Hours	Number					5
	Deaths					0
	Mortality					0

(*c*) the gradual rise in mortality in the Achromycin-treated animals as occlusion was prolonged, so that there was a high mortality (75 per cent) after 2 hours and 10 minutes of occlusion.

In three untreated dogs not included in the survival statistics, blood cultures were taken under sterile conditions from the portal vein before, during, and immediately following simultaneous occlusion of the celiac and superior mesenteric arteries for 1 hour and 30 minutes. Five cubic centimeters of blood was mixed with 20 cc. of nutrient agar and incubated for six weeks. Portal vein blood was sterile before occlusion in each case. In two out of three dogs there appeared two colonies at the end of 45 minutes, increasing to five colonies after 1 hour and 30 minutes before releasing the clamps. The third dog's portal vein blood remained sterile until release of the clamps, after 5 minutes of flow a culture grew out three colonies. The

other two dogs had an increase in colonies to seven each 5 minutes after release of occlusion The organism isolated in all cases was *Clostridium chauvoei*, no other was found

DISCUSSION

There are a number of possibilities to be considered as the cause of death from lower thoracic aortic occlusion. These include renal damage, liver ischemia, intestinal ischemia with or without absorption of bacteria through the bowel wall into the portal blood stream, and lastly the possibility of death being due to a mechanism similar to tourniquet shock.

It seems unlikely from these and other experiments that renal damage is an important cause of death It has been shown that it is necessary directly to occlude the renal arteries for two hours before significant kidney damage occurs.[10] It is well known that the kidneys can be completely removed, with survival for several days or weeks.

Although it is felt by some[6] that reduction of the oxygen supply to the liver is an important cause for the development of irreversibility to transfusion in hemorrhagic shock, Markowitz and his colleagues[9] have shown

Table 2 Mortality from Simultaneous Occlusion of the Superior Mesenteric and Celiac Arteries

		1 HOUR	1 HOUR 15 MINUTES	1 HOUR 30 MINUTES	1 HOUR 45 MINUTES	2 HOURS 10 MINUTES
Controls no premedication	Number	5	8	10	10	4
	Deaths	1	3	7	9	4
	Mortality	20%	37 5%	70%	90%	100%
Achromycin premedication	Number			10	10	4
	Deaths			1	4	3
	Mortality			10%	40%	75%
Penicillin premedication	Number			10		
	Deaths			3		
	Mortality			30%		

that the liver can be completely deprived of its regular arterial blood supply and yet survive on its collateral circulation Experiment B in our studies, wherein 60 per cent of the animals died after 1 hour and 30 minutes of occlusion of the aorta with maintenance of the normal arterial supply to the liver, appears to be good evidence that hepatic ischemia is not the lethal factor. It might be argued that in this instance, with flow through the superior mesenteric artery impaired, the liver suffers from reduction in its oxygen supply, through curtailment of portal vein flow This reduction is no different from and no greater than occurs with an Eck fistula We therefore conclude that liver damage from ischemia is not the cause for the shocklike state.

The maintenance of flow through the vessels of the gastro-intestinal tract, even though the hepatic artery was occluded, reduced the mortality markedly, even after 2 hours of clamping, to 10 per cent (experiment C, Table 1). Nevertheless, if the clamping time was increased to 2½ hours, all the animals died It was apparent that ischemia of the gastro-intestinal tract played an important role in the cause of high mortality. It was not the sole cause of death, as indicated by the high fatality rate after two hours and thirty minutes with the superior mesenteric artery open Fine and his co-workers[6]

have studied the transmural migration of intestinal bacteria during ischemic states, and have implicated this process as important in the mortality following prolonged hemorrhage. Our studies, as outlined in Table 2, indicate that preliminary sterilization of the gut definitely decreases the mortality from intestinal ischemia, despite the fact that we were unable to culture any organism other than *Clostridium chauvoei* from the portal vein blood. Here again the bacterial factor is not the only factor, as sterilization only reduced the mortality but did not prevent death entirely. It allowed prolongation of occlusion time, but a high mortality still occurred if the intestinal vessels were clamped long enough.

In summarizing the data from all of these experiments, therefore, we can arrive at only one logical conclusion: the mortality rate for a given period of aortic occlusion is directly proportional to the amount of vascular bed rendered ischemic. Undoubtedly hepatic anoxia, renal ischemia, and bacterial absorption from the intestine are contributory factors, but it seems more likely that the primary cause of death from occlusion of the lower thoracic aorta is a consequence of ischemia of the huge vascular bed comprised of the liver, intestines, kidneys, spleen, pelvis, and legs. This syndrome is analogous to tourniquet shock.

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PRODUCTION OF HEART BLOCK IN DOGS, UNDER DIRECT VISION*

MANSUR TAUFIC, FOUAD A. BASHOUR, AND F. JOHN LEWIS

The advent of surgical procedures for the repair of congenital defects of the cardiac septa brings up, among other problems, the matter of traumatic heart block. Is the trauma of reparative surgery in this area likely to cause heart block? More than theoretical considerations have turned our attention to the question, for we found that complete heart block frequently followed the experimental production, with a punch, of high ventricular septal defects.[13] Of course, such an operation, which actually removes tissue, is apt to be more injurious than a repair; but it did show that surgery in the high septum, where congenital defects occur, could result in heart block. Anatomically this area is, in its relationship to the conducting system, just beyond the auriculoventricular bundle, and, therefore, just beyond the region where complete heart block is easiest to produce. Still, as these experiments will show, it is possible without removing tissue but with a precisely localized injury to produce complete heart block.

Despite some controversy,[5] it is generally accepted that complete interruption of the auriculoventricular pathway will result in heart block. In dogs, this interruption of the bundle of His has been brought about by several different types of trauma.[2,4,6,8,11] In the experiments to be described, complete and permanent heart block has resulted from cutting or ligating the portion of the ventricular septum, beyond the bundle of His, where the initial segments of the right and left bundle branches are located.

ANATOMIC CONSIDERATIONS

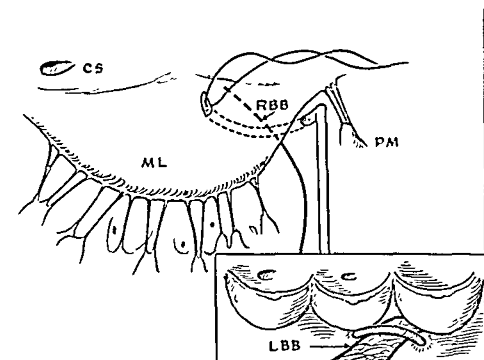
Recent anatomic study[1] has demonstrated that the auriculoventricular conducting system of the canine heart is a definite morphologic entity which can be identified either histologically or by gross dissection. The course of the two bundle branches can be followed on the surfaces of the ventricular septum by the naked eye, and when the septal endocardium is treated with an iodide preparation these branches stand out. The right bundle branch, when entirely subendocardial, appears like a discrete narrow band crossing under the anterior edge of the septal leaflet of the tricuspid valve and running toward the base of the anterior papillary muscle. The left bundle branch, which is constantly subendocardial, originates beneath the anterior half of the posterior aortic cusp. Initially it is like a broad and flat sheet, but after a short distance it fans out abruptly toward the apex of the septum and the base of the anterior and posterior papillary muscles of the mitral valve. The first portions of both the right and the left branches are located in the same region of the ventricular septum This is the region which has been injured and it is immediately below the commissure between the right and the posterior aortic cusps on the left ventricular side, and on the right

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side it is underneath the anterior portion of the medial leaflet of the tricuspid valve.

METHODS

Thirteen adult mongrel dogs of various sizes were operated on. In two of these animals the experiments were performed under general hypothermia, while the remaining dogs were operated upon at normal body temperature under intravenous Nembutal anesthesia. There was no difference between



[illegible] conus, *RBB*, right bundle branch, *LBB*, left bundle branch.

the response of the two cold dogs and the eleven normothermic dogs.

Before opening the chest, electrodes were placed in the positions suggested by Kimura[9] and electrocardiograms were taken before, as well as during and immediately after surgery with the animal lying in the left lateral position

The chest was entered through the right fourth interspace and the pericardial sac was opened anterior and parallel to the phrenic nerve. After tying the great azygos vein, both venae cavae were occluded temporarily with loops of heavy silk, and the aorta and pulmonary artery with a Satinsky clamp. The right atrium was then opened and a retractor was applied to the anterior leaflet of the tricuspid valve through the atrial incision in order to expose the tricuspid area of the ventricular septum.

To produce heart block two different techniques were used. In a series of 8 animals, a straight hemostat crushed the portion of the ventricular septum where the two bundle branches are located. In addition, an incision of 1.0 to 1.5 cm was made just below the hemostat. With this procedure

a linear interventricular septal defect was produced. In another series of 5 dogs the portion of th[illegible]m containing the branches was ligat[illegible] a small, sharp pointed aneurysm [illegible]ttened the myocardial tissue was [illegible] The endocardial layers were not torn by the liga-t[illegible], consequently, an interventricular communication was not produced. Frequently, however, the medial leaflet of the tricuspid valve was included *in the ligature* (Fig. 1).

Once the intracardiac procedure had been performed the right chambers were filled with saline, the atrial incision was closed with a Glover clamp, and the circulation was restored If heart block was not produced, one or



FIG 2 *a*, Left ventricular aspect of a canine heart specimen showing an interventricular septal defect resulting from the crushing and section technique for the production of heart block *b*, Electrocardiogram of the same dog on the second postoperative day

two subsequent attempts were made before suturing the atrial incision. Finally, the atrial incision was closed with a double layer of continuous fine silk suture and the clamp was removed The time of caval occlusion averaged 2 minutes and 30 seconds.

Electrocardiograms were taken daily in the first week and periodically thereafter.

The surviving animals were sacrificed early in the postoperative period or three months after surgery. Postmortem studies of the heart were limited to gross examination.

RESULTS

All 8 animals in which the crushing and cutting technique was carried out

had complete heart block. In 2 of these dogs the condition was induced only after a second attempt. There were 4 operative deaths in this series, caused primarily by other conditions associated with heart block, such as cardiac failure due to a large interventricular septal defect, aortic insufficiency due to injury of the aortic valve, irreversible ventricular fibrillation, and hemorrhage from the cardiotomy. There were no long-term survivors in the series of the 4 dogs which survived surgery. One dog died on the fourth postoperative day from pulmonary edema. Two days after surgery this animal began having epileptiform seizures typical of the Stokes-Adams syndrome and at that time electrocardiograms showed frequent periods of prolonged



Fig. 3. *a*, Left ventricular aspect of a heart specimen from a dog sacrificed three months after the production of heart block by the ligation technique. The ligature, covered by scarred endocardium, is seen at the *critical area* *b*, Electrocardiogram of the same dog.

ventricular asystole (Fig. 2) The remaining 3 animals died from pulmonary edema on the second, third and seventh days after surgery, respectively. At autopsy it was found that the septal incision transected both conducting branches in every case.

Among the 5 animals in which the ligation technique was carried out, complete heart block occurred in all but one dog. In this animal three attempts to produce heart block were unsuccessful. When this dog was sacrificed on the thirteenth postoperative day, examination of the septum showed that the ligatures had included the left bundle branch only partially. The only death in this group was caused by ventricular fibrillation which did not respond to electrical shock There were 3 survivors with complete heart block; one was sacrificed early in the postoperative period and two were

a linear interventricular septal defect was produced. In another series of 5 dogs the portion of the ventricular septum containing the branches was ligated with a stitch of heavy silk carried by a small, sharp pointed aneurysm needle. When the ligature was snugly tightened the myocardial tissue was transected by the thread The endocardial layers were not torn by the ligature; consequently, an interventricular communication was not produced Frequently, however, the medial leaflet of the tricuspid valve was included in the ligature (Fig. 1).

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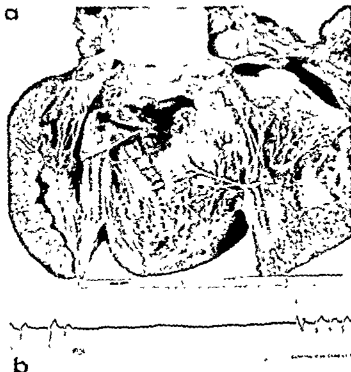


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sacrificed three months after surgery. The heart specimens of these long-term surviving animals showed pronounced myocardial hypertrophy with dilatation of both ventricles. In these two instances the ligature was found to be covered by a thick scar and it included both bundle branches.

DISCUSSION

In our study the ligation method for blocking the conducting system at the level of the bundle branches proved to be superior to that of crushing and section. The ligature caused less trauma and prevented the simultaneous and unfavorable creation of an interventricular septal defect

Observations made by other investigators[3, 4] regarding some major characteristics of experimental heart block produced by complete transection of the conducting system were confirmed in the present experiments. Thus, heart block was always complete and permanent In no instance did chronic heart block revert to normal.

When heart block occurred in these experiments the injury was localized in a specific area of the high septum. This is the same area where we had previously found that a punch defect might often cause heart block.[13] Other parts of the ventricular septum are not as sensitive. We found that defects created in other locations, such as the pulmonary infundibulum, did not cause heart block [14] In comparative anatomy, the *critical area* here discussed corresponds, in man, to the *pars membranacea septi* which is closely related to the bundle of His and the initial segments of the branches Since congenital interventricular septal defects in humans occur most frequently at the membranous septum,[12] suture repair of these lesions may not be as innocuous as it has been in the repair of most interatrial septal defects.[10] While the interatrial septum is largely insensitive, it is not entirely so, for during the suture repair of two septum primum defects of the atrial septum in man we have produced complete heart block in both. In fact, it may be easier to cause heart block while repairing this type of defect than it is while operating in the ventricular septum, for with a septum primum defect there is danger of injuring the main auriculoventricular bundle rather than the separate right and left branches.

SUMMARY

1. Two procedures were employed to cause complete heart block in normothermic as well as in hypothermic dogs In 8 animals the septum was simultaneously crushed and severed, in 5 other dogs a ligation technique was used.

2 Complete heart block was produced in 12 of these 13 animals

3. Postmortem examination showed the lesion to be located in that portion of the ventricular septum crossed by the initial segments of the right and left bundle branches.

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AN EVALUATION OF THE USE OF AN ELECTRICAL PACEMAKER TO MAINTAIN THE FUNCTION OF THE HEART OF THE HYPOTHERMIC DOG*

H. BRODIE STEPHENS, ROBERT H. DE RIEMER, AND RICHARD WEXLER

With the increasing frequency of cardiac surgery, there appears to be an increase in the occurrence of cardiac arrest which obligates the surgeon to investigate further means to meet and understand this emergency. Cardiac standstill, or asystole, though it accounts for the majority of instances of cardiac arrest, has not received the extensive study which the problem of ventricular fibrillation has. One of the difficulties has been the inability to produce cardiac standstill experimentally under conditions of apparently adequate oxygenation and in an animal with the capacity of cellular response. It has been shown by Callaghan and Bigelow[1] that the heart of the hypothermic animal responds to electrical stimulation following induction of cardiac standstill with low body temperatures.

Similar work carried on in this laboratory is presented to further evaluate the effectiveness of electrical stimuli in re-establishing and maintaining the cardiac output in the hypothermic dog.

METHOD

Medium-sized mongrel dogs were completely shaved and anesthetized

* From the Surgical Research Laboratories of the University of California School of Medicine, San Francisco. This study was supported by the Christine Breon Research Fund.

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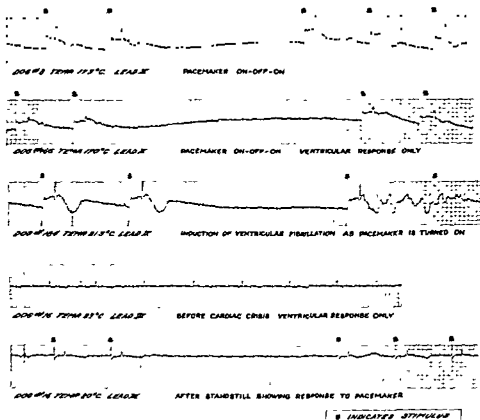


Fig. 1. Electrocardiograph tracings showing responses to electrical pacemaker with electrode located adjacent to the sinoauricular node.

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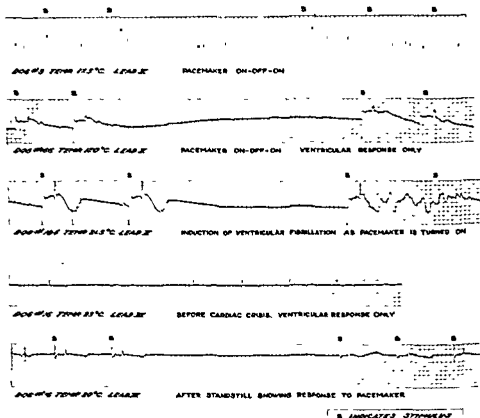


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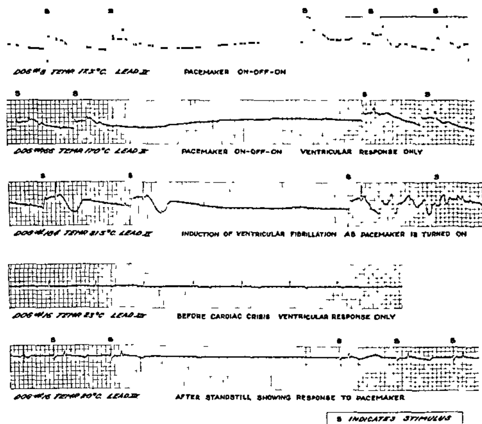


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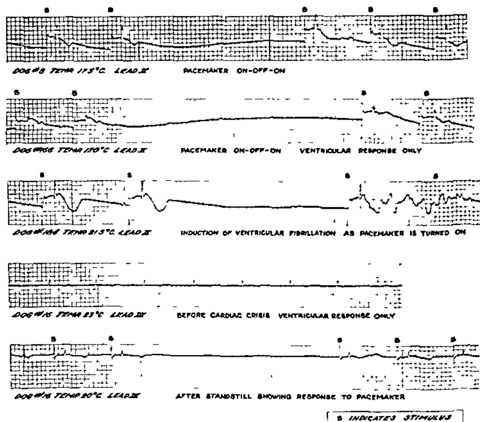


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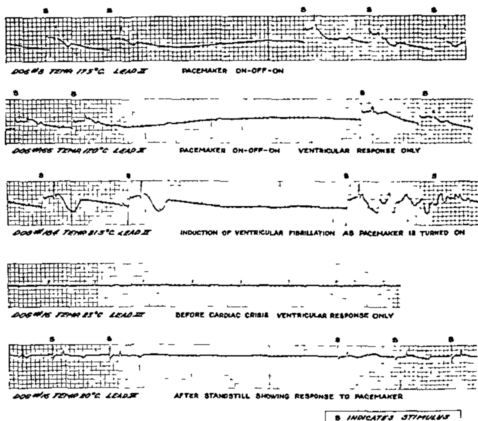


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Table 1. Blood Pressures, Pulse Rates and Rectal Temperatures in Dogs at Normal Temperature, Preceding Cardiac Crisis and While Being Maintained on Pacemaker

DOG NO	BASELINE			DURATION OF COOLING (MIN)	JUST PRECEDING CARDIAC CRISIS			NATURE OF CRISIS	ON PACEMAKER		NO OF MIN. ON PACEMAKER
	BP	PULSE	T(°C)		BP	PULSE	T(°C)		BP	PULSE	
136	150/105	140	37	80	100 55	42	23	Standstill	105/65	36	45
30	170, 120	168	37	90	80 65	44	23	Standstill	67/50	48	32
88	170/100	162	37 5	137	60/30	24	20	Vent fib	(Fibrillation induced deliberately. Failed to respond)		
95	170/110	160	38	89	80 60	36	21	Standstill	70/45	30	84
166	145/100	132	39	122	60, 10	36	20	Vent fib	60, 25	36	126
16	170/110	128	38	152	52 35	30	21	Standstill	50 35	30	75
185	160 110	144	37	98	80/35	24	21	Vent fib	(Pulse very slow Placed on pacemaker, inducing vent fibrillation)		
108	145 95	120	38	60	125/95	72	27	Vent fib	(Given Prostigmin intramuscularly prior to cooling)		
8	130/80	144	39	128	65 45	30	20	Standstill	75/45	36–30	110
184	155/100	130	37 7	78	55/20	15	23	Standstill	50 15	15	12
134	140/80	108	37	35	135, 90	72	25	(Ventricular fibrillation when chest was opened)			

hypothermic heart in standstill occasionally had random ventricular complexes or showed no response at all. While the animals were responding to the pacemaker, electrocardiogram tracings were obtained demonstrating the stimulus, followed by a P wave and the QRS complex. The contraction wave could be observed visibly arising in the auricle, and spreading out over the ventricle for systole. Occasionally only a ventricular response was obtained while maintaining the heart on the pacemaker, and in these animals no auricular response could be observed in the heart or by electrocardiogram. Esophageal leads were obtained during stimulation to demonstrate that the P waves did not represent artifacts from the stimulus.

It was possible to elevate the rate of both the normothermic and the

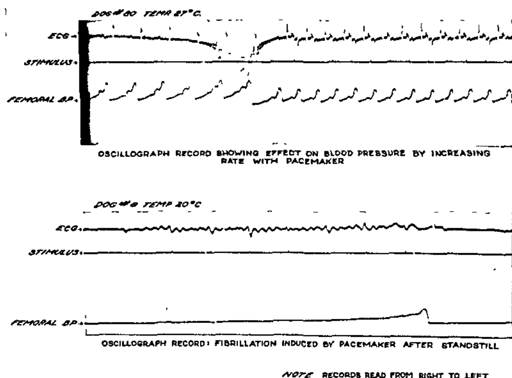


Fig 2. Oscillograph tracings demonstrating effect of pacemaker on animal with spontaneous heartbeat and animal in ventricular fibrillation.

hypothermic animal using stimuli of the same voltage and duration by increasing the frequency of the electrical stimulus. However, any increase in the rate was made primarily at the expense of the relaxation phase electrocardiographically and by gross observation. Pressures recorded during this artificial increase in rate showed a drop in systolic pressure but not in the diastolic pressure The mean pressure remained approximately the same as that while the animal was maintaining his own rhythm (see Fig. 2). As the animals were further cooled while being maintained on the pacemaker it was necessary to decrease the frequency of the stimulus, or the heart would appear to have inadequate filling and would not respond to each stimulus In these experiments it was not possible to produce effective slowing of the cardiac rate by means of the electrical stimulator in normothermic animals or in hypothermic dogs that had not yet developed cardiac arrest.

The polarity of the electrical stimulus had no apparent effect upon its effectiveness and it produced no further changes in the electrocardiographic response. Use of a biphasic stimulus of the same voltage and duration as for a monophasic pulse failed to produce any response.

During prolonged maintenance of the cardiac rhythm by means of the pacemaker, frequent observations were made by turning the stimulator off and then on again. These recordings showed complete absence of electrical activity and a fall in blood pressure or infrequent ventricular responses only. Ventricular fibrillation occasionally resulted as the electrical stimulus was re-instituted, necessitating electrical defibrillation before the stimulus was effective.

Five of the animals which responded to the artificial pacemaker eventually developed intractable ventricular fibrillation. The remaining animal showed decreasing responsiveness to the pacemaker and expired with marked dilatation of the heart.

SUMMARY

1. An electrical stimulus of 1½ to 2 volts of 2 to 10 milliseconds duration has been used as an artificial cardiac pacemaker in the dog. It was possible to maintain cardiac rhythmicity and blood pressure in arrested hypothermic hearts in 6 of 11 dogs for varying periods of time.

2. Records are presented demonstrating the nature of some of the responses of hypothermic dogs to such a pacemaker.

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THE EFFECT OF CARBON DIOXIDE ON VENTRICULAR FIBRILLATION AND HEART BLOCK DURING HYPOTHERMIA IN RATS AND DOGS*

SUAD A. NIAZI AND F. JOHN LEWIS

Cardiac arrhythmias in the form of heart block, and ventricular fibrillation have been noted during hypothermia by many observers,[1-7] and these complications have, in fact, been obstacles when using hypothermia to perform intracardiac surgery. In hypothermic rats and dogs, we noted heart block

* From The Department of Surgery, University of Minnesota Medical School, Minneapolis. This investigation was supported by Research Grant H-1374 of the National Heart Institute of the National Institutes of Health, Public Health Service, and by Grants from the University of Minnesota Graduate School and the Minnesota Heart Association.

when the body temperature dropped below 20°C., but this complication has been prevented and also successfully treated by adding carbon dioxide to the inspired air or oxygen. Ventricular fibrillation was not noted in rats cooled according to our method,[8] but it was a common hazard in hypothermic dogs, and this complication, too, has been significantly reduced by adding carbon dioxide to the inspired air or oxygen.

METHODS

Adult Sprague-Dawley rats, and young mongrel dogs, 3 to 6 months old, were cooled during Nembutal anesthesia, in blankets through which circulated a solution of 50 per cent alcohol in water, cooled to between −6° and −12°C. Atropine was given 30 minutes [illegible] rats, artificial respiration was used during [illegible] effect of oxygen or air and mixtures of thes[illegible] tested. Rewarming was performed with wet packs heated to 45°C. Electrocardiograms were taken during the experiment with a direct writing machine. The temperature of the animal was measured by a mercury thermometer introduced 8 to 9 cm. into the rectum of the rat and 10 to 15 cm. in the dog.

RESULTS

Heart Block in Rats. 1. When 10 rats were cooled without artificial respiration, heart block appeared as their temperature dropped below 20°C., and their breathing gradually became imperceptible. All of these rats succumbed during rewarming even if artificial respiration was finally used during rewarming In two of these rats heart block disappeared when artificial breathing was used and returned when it was discontinued.

2. Artificial respiration was used during the cooling of 13 rats. Four of these were hyperventilated at a rate of 72 per minute with air or oxygen and the remainder had a respiratory rate of 10 to 32 per minute. All of these rats developed heart block. It occurred at about 19°C. in the rats with hyperventilation and at around 10°C. in those with the slower respiratory rate The temperature at which cardiac standstill occurred fell also, from 14°C. in those with hyperventilation down to 8°C. in those with the slower respiratory rate

3 When 5 per cent carbon dioxide in oxygen was given to 5 rats and 20 per cent CO_2 to a sixth during cooling, no heart block was noted except when the CO_2 was discontinued. The heart block disappeared again when CO_2 was resumed In these animals which received CO_2 cardiac standstill did not occur and their hearts continued to beat even though they were cooled down to 0°C.

As we were interested, primarily, in the effect of carbon dioxide on heart block, rewarming was attempted in only 9 of the rats. Of these 9, two had been given 5 per cent CO_2, one had been given 20 per cent CO_2, two were hyperventilated during cooling and four had been cooled with a slower respiratory rate. Without exception, all of these rats survived.

[illegible] 1. When 10 dogs were [illegible] [illegible]oling, ventricular fibril- [illegible] of them. This occurred when the body temperature reached levels of 13° to 17° (average 14.6°). The hearts of the remaining 6 dogs continued to beat even though the

temperature fell down to between 0° and 7.5°C. (average 4.9°). Three of these dogs with continued heartbeat developed heart block. This occurred at 15° to 18°C. (average 16°).

Thus of 10 dogs which were given oxygen alone during cooling, 40 per cent developed ventricular fibrillation and 70 per cent of the group showed heart block.

2. When 2.5 to 11.4 per cent carbon dioxide was given with the inspired oxygen continuously during cooling in 11 dogs, ventricular fibrillation and heart block did not occur in any of them. Their hearts continued to beat down to between 0° and 8.5°C. (average 3.8°).

[illegible] ide in oxygen [illegible], discontinued [illegible] oxygen alone was then used for the rest of cooling and for the rewarming. Ventricular fibrillation preceded by heart block occurred in 4 of these dogs when their temperatures had fallen to between 15.5° and 19°C. (average 16.1°). Among the remaining dogs, cardiac standstill occurred in 8 at temperatures of 10° to 15.5°C. (average 13°), while the heart continued to beat down to between 3° and 6° in seven. Heart block was noticed in two dogs before cardiac standstill occurred, and in 3 among those that had continued heartbeat. Thus in 19 dogs, when carbon dioxide was given in the first part of cooling and then discontinued, ventricular fibrillation occurred in 21 per cent and heart block in 47 per cent.

Rewarming was attempted in the group of dogs described above. All 10 [illegible] up of 11 dogs breathing carbon [illegible] was high too, as only one dog [illegible] carbon dioxide was discontin[illegible]ued—six were in the group of eight that had cardiac standstill, and two were among seven dogs in which the heart continued to beat throughout the experiment.

DISCUSSION

Artificial respiration is apparently necessary in order to prevent heart block in rats cooled below 20°C. This is understandable, for at that temperature the spontaneous respiration becomes imperceptible and it is quite probable that the cardiac arrhythmia noted without artificial respiration was due primarily to anoxia. The picture becomes more complicated, however, when the observation is made that hyperventilation, as well as no artificial respiration, may lead to heart block. It may be that respiratory alkalosis is produced by hyperventilation and this may induce a deficit in tissue oxygen by shifti[illegible] Thus the two extremes of hypo[illegible] ssue hypoxia and cause cardiac [illegible] avoid heart block has been to give adequate amounts of oxygen plus carbon dioxide, thus avoiding both direct anoxia and alkalosis. No rats cooled in this manner developed heart block.

In dogs, too, heart block and ventricular fibrillation are prevented by adding carbon dioxide to the respired gas. The effect of carbon dioxide in [illegible]

not occur while carbon dioxide was being given but after it was discontinued ventricular fibrillation occurred in 21 per cent of the animals and heart block in 47 per cent. This incidence of arrhythmias is not as high, however, as it was in the animals cooled without receiving any carbon dioxide at all, even from the beginning of cooling; for among these, 40 per cent had ventricular fibrillation and 70 per cent had heart block.

*p*H determinations of the arterial blood in hypothermic dogs have shown striking and dangerous elevations if carbon dioxide is not used in the breathing mixture. When oxygen alone was given for breathing the *p*H would rise as high as 8.4 even without hyperventilation. The rise in the *p*H was more marked as the temperature dropped below 20°C., and the carbon dioxide content of the blood was found as low as 9 0 mM./L. at 10°C. The highest *p*H with survival was 8 1. When carbon dioxide was given this rise of the *p*H was avoided and the *p*H was either kept within normal limits or decreased.

The correlation of *p*H values with oxygen content of the blood and with oxygen consumption by the tissues at the lowered temperatures would be of great value in the clarification of this entire problem.

We feel at the present time that hyperventilation with oxygen alone should be avoided during hypothermia for cardiac surgery and that carbon dioxide, probably as 5 per cent in oxygen, should be used as the breathing mixture.

SUMMARY

1. In hypothermic rats and dogs, either hypoventilation or hyperventilation of room air or the breathing of oxygen alone results in a high incidence of cardiac arrhythmias.

2. To prevent these arrhythmias an adequate amount of both oxygen and a level of carbon dioxide approximating 5 per cent in the respired gas are necessary.

3. It is suggested that the basic cause of arrhythmias during hypothermia is hypoxia, whether this follows hypoventilation directly or whether it results as a consequence of alkalosis.

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VENTRICULAR FIBRILLATION IN HYPOTHERMIC DOGS*

CHARLES K. KIRBY, JAY M. JENSEN, AND JULIAN JOHNSON

Ventricular fibrillation is a frequent complication of the use of hypothermia for intracardiac surgery. It has been our impression that it is more difficult to abolish ventricular fibrillation by electric shock in hypothermic than in normothermic dogs. Others have thought that the difficulty was no greater [1] This problem has not been studied under controlled conditions, to our knowledge. These experiments were undertaken in an attempt to answer this question. In addition, the effects of Prostigmin methylsulfate and other drugs were studied

METHODS

During experiments recently reported[2] we developed a standardized technique for inducing and abolishing ventricular fibrillation in normothermic dogs. Fibrillation was induced by passing a current of 10 volts through the heart for 0 50 second. The heart was allowed to fibrillate for four minutes before institution of resuscitative measures. It was then compressed at a rate of 80 times per minute for two minutes, and the defibrillating shock was applied. Single, 0 10 second shocks were used. The strength of the initial shock was varied between 110 and 270 volts in different series of animals Increasingly stronger currents appeared to be increasingly effective. Single shocks of 220 and 270 volts were almost invariably successful. Most of the dogs survived until killed, and there was no evidence of myocardial burns at autopsy The results of these experiments with anoxic, normothermic hearts appeared to provide suitable controls for experiments with hypothermic hearts.

Group A [illegible] esthetized with intravenou [illegible] An endotracheal tube was [illegible] per cent oxygen The body [illegible] the dogs with chipped ice This usually brought the rectal temperature down to 26°C. within an hour. An additional 30 minutes was sometimes required to lower the temperature to 20°C. The temperature usually fell an additional 2° to 4°C after the ice was removed Allowance was made for this additional drop in planning the time of removal. The left chest was entered through the fifth or sixth intercostal space, with the dog in the lateral position, and the pericardium was opened widely Ventricular fibrillation was induced with a 0 50 second shock of 10 volts. The heart was allowed to fibrillate for four minutes and was then compressed for two minutes at a rate of 80 times per minute. The defibrillating shock of 220 volts was then applied for [illegible] apy was [illegible] another [illegible] d, serial 0.10 second shocks were applied after an additional minute of cardiac compression. If the initial shock abolished fibrillation but the heart went into standstill, it was observed for a period of 10 to 20 seconds to see whether

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it might begin to beat spontaneously. When it did not, the heart was compressed intermittently until it was beating on its own. Epinephrine hydrochloride (0.30 cc. of 1:1,000 solution) was injected into the right auricle if strong, spontaneous heartbeats did not resume after 15 minutes.

When it appeared relatively certain that continued effective spontaneous heart action could be anticipated, the chest wall was closed in layers. Oxytetracycline (Terramycin) was administered intravenously during the operative procedure. The animals were partially immersed in a basin of water at 45°C. for rewarming. The rectal temperature usually returned to 38°C within an hour. Postoperative care was routine and did not differ from that for other dogs in the colony. The animals were carefully observed during the first few postoperative days for evidence of cerebral or cardiac injury. They were sacrificed within 10 days after operation, and the hearts were examined carefully for evidence of burns or other injuries at autopsy. There were 35 dogs in this series.

Group B: Experiments with Prostigmin and Electric Shock. In 14 dogs, Prostigmin was used in the manner suggested by Dr. H. C. Swan,[3] who *believes it increases the likelihood that electric shock will cause defibrillation.* The procedure was the same as in group A except that prior to induc-

Table 1. Result of a Single Shock of 220 Volts for 0.10 Second

TEMPERATURE (CENTIGRADE)	NUMBER OF DOGS	DEFIBRILLATED. NORMAL HEARTBEAT	DEFIBRILLATED PERSISTENT ASYSTOLE	NOT DEFIBRILLATED
26°	20	10	6	4
24°	4	2	1	1
23°	4	2		2
22°	3	1	1	1
20°	4		1	3
Total	35	15	9	11

ing fibrillation the venae cavae were occluded, the aorta was cross-clamped just above the heart and 1 0 cc. of Prostigmin methylsulfate solution was injected into the aorta proximal to the clamp The injection was made slowly, over a 30 second period. Thirty seconds later the aorta clamp was removed. The heart was slowed to a rate of between 10 and 20 beats a minute in all instances. Two minutes later fibrillation was induced and the remainder of the experiment was the same as in group A.

RESULTS

Group A: Electric Shock Alone. The results are summarized in Table 1. In only 15 of the 35 dogs was the result essentially the same as in normothermic dogs, i e , prompt cessation of ventricular fibrillation and resumption of normal cardiac activity. There was usually a definite, observable period of asystole before the first contraction, however. This was in contrast to normothermic dogs, in which the first contraction usually followed the electric shock so closely that a period of asystole was not observable.

In 9 of the dogs, ventricular fibrillation was promptly abolished but the heart remained in asystole. After 15 to 20 seconds of observation, cardiac compression was begun. One or two compressions usually stimulated the occurrence of a strong ventricular contraction, but there was then another

period of standstill. Intermittent compression was necessary for at least 10 minutes in all of the animals. Epinephrine hydrochloride (0.030 cc. of 1:1,000 solution) was finally used in three of the animals because of persistent, weak contractions.

Of the 11 dogs not defibrillated by the test shock, a second shock was successful in three. Repeated shocks were necessary in the other eight. All were eventually defibrillated. Intermittent compression was necessary in several.

All of the animals survived the operation. Four died within 24 hours. The remainder lived until they were sacrificed. The hearts appeared grossly normal in most instances A few had areas of subendocardial ecchymosis, presumably as a result of the trauma of cardiac compression. In four instances of serial defibrillation, electrode burns were clearly visible.

Group B: Prostigmin and Electric Shock. The results are summarized in Table 2 Immediate defibrillation with effective heartbeat occurred in 9 of 14 dogs (64 3 per cent), in contrast to 15 of 35 (42 8 per cent) in which Prostigmin was not used. Inasmuch as the series is not large, it may not be justifiable to draw definite conclusions concerning the efficacy of Prostigmin

Table 2 Result of a Single Shock of 220 Volts for 0.10 Second after Prostigmin

TEMPERATURE (CENTIGRADE)	NUMBER OF DOGS	DEFIBRILLATED NORMAL HEARTBEAT	DEFIBRILLATED PERSISTENT ASYSTOLE	NOT DEFIBRILLATED
26°	10	7		3
24°	4	2	1	1
Total	14	9	1	4

COMMENT

It is evident that, under the conditions of these experiments, ventricular fibrillation was somewhat more resistant to the effects of electric shock in hypothermic than in normothermic dogs. In addition, the abolishment of ventricular fibrillation was often followed by a period of cardiac arrest which was difficult to overcome This never occurred in animals which had not been cooled Persistent asystole has also apparently been a problem in the experiments of Hopps and Bigelow,[4] who have used an electrical pacemaker until the heart is again beating spontaneously.

The hypoxia resulting from a four minute period of fibrillation should, theoretically, have been less severe in the hypothermic dogs. This may be further evidence that the hypothermic state *per se* increases the difficulty of defibrillation

CONCLUSIONS

1. It was more difficult to abolish ventricular fibrillation by electric shock in hypothermic than in normothermic dogs.
2. Persistent asystole was a problem in many hypothermic dogs after ventricular fibrillation was stopped
3. Defibrillation by electric shock appeared to be slightly easier when the

coronary arteries were perfused with Prostigmin methylsulfate prior to the induction of fibrillation.

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RESUMPTION OF HEARTBEAT IN DOGS AFTER STANDSTILL AT LOW TEMPERATURES*

SUAD A. NIAZI AND F. JOHN LEWIS

Moderate hypothermia has permitted total circulatory interruption for periods of time sufficient for the performance of successful intracardiac operations under direct vision[1-6] However, the limited time of circulatory occlusion which is tolerated still remains a drawback which can only be removed if deeper levels of hypothermia are possible.

Previous investigators have not been able to drop the temperature of nonhibernating mammals below 13° to 20°C. But in cooling rats,[7] we found that deep levels of temperature tolerated only by hibernators could be reached In dogs, too, we have found that the body temperature could be dropped to levels below 10°C. and as low as 2°C. with survival. Dogs, as in rats, passed through the lower temperature ranges in a state of temporary cardiac and respiratory arrest which might last for more than one hour with suvival.

METHODS

Mongrel dogs weighing from 8 to 19 pounds were cooled in blankets through which 50 per cent alcohol in water at −12°C. circulated in rubber tubing. The age of the dogs varied from 2 months up to 2 years. Atropine (1/100 grain) was given before cooling was begun. Artificial respiration was started with the cooling and 5 per cent carbon dioxide in oxygen was given for breathing until the temperature of the dog had dropped to 20°C., when carbon dioxide was discontinued and oxygen alone was given until the heart stopped beating. Respiration was stopped during the period of cardiac standstill and started again with oxygen when the dog was rewarmed. Rewarming was achieved with hot wet packs at 45°C. which were applied to the chest until the heart had started to beat regularly. Then, rewarming of the whole body was undertaken by pouring water at 45°C on wet packs covering the animal. Electrocardiograms were taken during

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Institut[illegible]
the Un[illegible]

the experiment with a direct writing machine. The temperature of the animal was measured by a mercury thermometer introduced 15 to 20 cm. into the rectum.

RESULTS

Twenty dogs were cooled by the method described. Ten (50 per cent) had cardiac standstill when their temperature reached 9° to 14.5°C. (average 12°) but the bc
(average 8.5°) In
standstill. Six (30 pe
0° to 8.5°C. (avera
tion occurred at 12 to 20°C. (average 16.6°).

On rewarming the 10 dogs which reached the desired objective of cardiac standstill, 8 (80 per cent) survived after periods of cardiac standstill lasting 1 to 1½ hours. These dogs had, in addition, a slow nodal or idioventricular rhythm—one beat every 1 to 5 minutes—before the heart was arrested, and again during rewarming before regular beats were regained. The total period of this slow rhythm was from 1 to 2 hours.

The surviving dogs recovered consciousness within ½ to 6 hours after normal temperature was regained. They were able to walk and eat within the next 3 to 6 hours. They have lived from 3 weeks up to several months.

With two exceptions, the survivors have appeared to be normal in every respect. The third and fourth survivors showed a temporary flaccid type of paralysis of their hindlimbs. In these dogs the whole trunk including the back was rewarmed at the same time, from the start, and it was about 30 minutes before the heart started beating. When the region of the vertebral column was excluded, at the beginning of rewarming, and the back was left in contact with the cooling blanket until regular heartbeat returned, this neurologic complication was avoided.

Rewarming was also performed in the dogs in which the heart continued to beat. There were 6 dogs in this g

When ventricular fibrillation occu
lation was attempted in three of the
heart was successfully defibrillated by local rewarming of the heart with saline at 38°C., intracardiac epinephrine, massage, and short electric shocks of 60 cycles and 100 volts. One dog lived 9 days after defibrillation was accomplished at 15°C. and died then of empyema and massive infection of the chest wall. Another dog lived 12 hours after the heart was defibrillated at 12°C., and the third, which was defibrillated at 18°C., died when the temperature reached 30°C.

DISCUSSION

To achieve temperature levels below 10°C. in dogs and in rats it is important to produce cardiac standstill during the cooling process. Continued heartbeat at the lower temperature levels results in a high mortality and the occurrence of ventricular fibrillation prevents further cooling.

In a previous study[8] we noticed that cardiac standstill did not occur when only oxygen was given during cooling, or when carbon dioxide was added and continued throughout the experiment. But when carbon dioxide was discontinued in the latter part of cooling, cardiac standstill occurred in about 50 per cent of the dogs. The method of giving 5 per cent of carbon

dioxide in the respired oxygen during cooling until the temperature has fallen to 20°C. and continuing oxygen alone for the rest of the experiment seemed to produce a higher incidence of cardiac standstill than other concentrations of carbon dioxide or when carbon dioxide was discontinued at temperature levels above or below 20°C. No dog has survived if concentrations of carbon dioxide of 10 per cent or higher were given during cooling, except one in which the 10 per cent carbon dioxide was discontinued at 25°C., and cardiac standstill has not been achieved when less than 3 per cent carbon dioxide was given during the early cooling period.

The method of producing cardiac standstill is not clear as yet. It does seem to be related to the reaction of the blood, however. We found that a high *p*H of 7.7 to 8.14 was present at the time of cardiac standstill. When carbon dioxide was continued throughout the experiment such high levels of *p*H did not occur and cardiac standstill was absent too. However, the same high *p*H levels at which cardiac standstill occurred were present when oxygen alone was given from the beginning of cooling, but in these animals cardiac standstill did not occur. A shift from a low to a high *p*H [illegible] 20°C. seems to be important in attain- [illegible] hift may produce ventricular fibrillation instead, in some cold dogs, as it does in dogs at normal temperatures.[9] Further work is being directed toward the production of a higher incidence of standstill, since this seems to be the safest trail to profound hypothermia.

SUMMARY

1. Cardiac standstill occurred in 50 per cent of dogs cooled to 0° to 11.5°C. and 80 per cent survived intervals of standstill lasting 1 to 1½ hours.

2. Ventricular fibrillation or continued heartbeat occurred in 20 per cent and 30 per cent of these animals, respectively, and among these the mortality was high

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THE EFFECT OF TOTAL ADRENALECTOMY UPON CARDIAC OUTPUT IN DOGS WITH LARGE CHRONIC ARTERIOVENOUS FISTULAS*†

A Comparison of the Hamilton Dye-Dilution Method and the Direct Fick Procedure under Experimental Conditions of Sustained Cardiovascular Stress

MITCHELL W. SPELLMAN, GABRIEL G. NAHAS, AND C. WALTON LILLEHEI

In previous studies[1] it was found that dogs with large arteriovenous fistulas were remarkably susceptible to the occurrence of a fulminating and usually fatal bacterial endocarditis following the intravenous introduction of bacterial organisms in numbers completely innocuous to dogs with a normal circulatory system

Further observations[2] upon the mechanism of this observed increase in cardiac valvular susceptibility to infection have disclosed that total adrenalectomy in these animals with bilateral arteriovenous fistulas completely protected them against the development of bacterial endocarditis when the substitution therapy consisted of Doca only. In similar dogs with total adrenalectomy and with bilateral arteriovenous fistulas, but in which maintenance therapy consisted of cortisone and supplementary sodium chloride, the cardiac susceptibility to vegetative bacterial endocarditis appeared to be essentially unchanged from that observed in the dogs with intact adrenals and similar vascular shunts.[3]

Pertinent to these experimental observations it appeared important to evaluate the influence of total adrenalectomy upon cardiac output in dogs maintained upon Doca only and upon cortisone and sodium chloride. Such studies have not been made previously.

Two different methods of measuring the cardiac output were employed nearly simultaneously in each animal, one, the basic Fick principle,[4] the other, the Hamilton dye method[5] modified by introducing the dye directly into the pulmonary artery

METHODS

Healthy large adult mongrel dogs of either sex were employed for this study The weights of the animals varied from 11.5 to 22 kg. Their diet consisted of commercial dog biscuits supplemented daily with one-half pound or more of raw horsemeat The adrenalectomized dogs received additionally 500 cc to 1000 cc of whole milk daily to insure an adequate diet

Following acclimatization of the animal to the colony, side to side arteriovenous fistulas 20 to 30 mm. in length were constructed during one stage

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† A portion of a thesis submitted to the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the Ph D Degree (Surgery).

between the iliac artery and vein on one side and between the femoral artery and vein in the opposite leg.

One month following the construction of the arteriovenous fistulas, the cardiac output was measured in 16 dogs utilizing both the dye and Fick methods in nearly all of these animals. Following these determinations, a bilateral total adrenalectomy was performed in two stages, usually 4 to 7 days apart, and the totally adrenalectomized dogs with bilateral arteriovenous fistulas were maintained thereafter as indicated below on either cortisone with supplementary sodium chloride or upon Doca alone.

Measurement of Cardiac Output.

All animals were anesthetized with sodium pentobarbital (Nembutal) 15 to 20 mg per kilogram of body weight for the cardiac output measurements. In most all of these animals both the dye dilution and the direct Fick method were employed on the same animal as nearly simultaneously as possible in order to increase the accuracy of the observations as well as to provide valid data for comparison of the two methods.

Briefly described, the dye-dilution method, as utilized here, consisted of the rapid injection of a known quantity of Evans blue dye (T-1824) into a cardiac catheter placed in the pulmonary artery near [illegible]
second catheter in the aorta. [illegible]
the aortic valve. The blood fr[illegible]
ing at a known velocity on a [illegible]
in the pulmonary artery is a modification employed by Ebert[6] and the adoption of the aortic collection catheter and the revolving tubes was proposed by Haddy and Baronofsky.[7]

After the arterial samples were analyzed colorimetrically, the serum concentration of the dye in milligrams per liter was calculated for each tube. The dye-dilution curve was then plotted, after the method of Hamilton, so that the abscissa (time in seconds) was a linear component and serum dye concentration (mg/L), a logarithmic component. With this procedure, the quantity of dye on its first circulation is measured by prolongation of the descending limb to the base line. The average concentration of the dye on its initial circulation was calculated from this curve, employing the sum of the average heights of the ordinates at one-second intervals. One can then calculate the cardiac output with the aid of the average dye concentration by using Hamilton's formula.[8]

Where the direct Fick procedure and the dye-dilution method were employed virtually simultaneously, the following technique was utilized. The oxygen consumption was measured over an eight-minute timed interval with the aid of an endotracheal tube and the Benedict-Roth spirometer. At the mid-point of this interval, samples of blood were simultaneously withdrawn from the venous catheter (in the pulmonary artery) and arterial catheter (in the aorta). The arteriovenous oxygen concentrations (of these samples) were measured by the gas analysis method of Van Slyke and with these arteriovenous oxygen differences and the oxygen consumption known, the cardiac output was calculated after the method of Fick.[4]

It should be mentioned for clarity that the cardiac output meas[illegible]
dilution method occurred approximate[illegible]
samples for the direct Fick procedure [illegible]
animals maintained upon Doca to surv[illegible]
repetition of the postadrenalectomy measurements [illegible]

Maintenance Therapy of the Totally Adrenalectomized Dogs.

The maintenance of adrenalectomized dogs with bilateral iliac and femoral arteriovenous fistulas, with or without an associated bacteremia, was a task requiring considerable care. The design of the experiment required that only a single [illegible]
sho[illegible]
pre[illegible]
sor[illegible]
[illegible] of each animal with measurement of the body temperature, pulse rate, observation of appetite and fluid intake, activity and strength combined with

urinary output were found to be reliable methods in the dog for clinically evaluating the adequacy of their substitution therapy. The periodic measurement of the blood urea nitrogen provided another sensitive indicator of the presence of adrenal insufficiency.

In the group of seven cortisone-treated totally adrenalectomized dogs with large arteriovenous fistulae in their hind extremities, the daily doses of cortisone varied from 25 mg. to 100 mg., although no such wide difference occurred in a single animal. On a weight basis, the minimum daily requirements were 1.3 mg./kg. body weight and the maximum, 5.6 mg./kg. Between these extremes, the usual daily doses varied from 2.6 to 4.8 mg. of cortisone per kilogram of body weight. In four of seven cortisone-maintained animals, the daily doses for the most part were of the magnitude of 2.6 to 2.8 mg. of cortisone/kg. body weight.

The usual daily supplementation of salt administered to each of the cortisone-treated totally adrenalectomized dogs was two to four grams beyond their dietary intake. Require-

Table 1. Measurement of Cardiac Index Employing the Dye-Dilution and Fick Methods Nearly Simultaneously in Dogs with Large, Chronic, Bilateral† Arteriovenous Fistulas*

DOG NO	CARDIAC INDEX DYE	CARDIAC INDEX FICK
1200	10.4 L	Not Done
1543	9.4	6.6‡
1644	10.0	Unsatisfactory Technique
1675	13.0, 10.8§	14.0
278	14.5, 13.3§	19.2
172	12.2	22.0
1201	13.0	Not Done
1749	10.4	13.0
1113	10.8	Not Done
1751	10.0	18.5
105	10.6	8.9
277	15.5	18.2
289	10.0	16.6
1106	9.8	16.2
332	9.5	9.2
592	10.6	24.5
Mean	11.1	16.4
Standard Deviation	1.71	4.65

* Cardiac Index = Cardiac output in liters/M² body surface/minute.

† Between the iliac artery and vein on one side, and the femoral artery and vein in the other leg.

‡ Value disregarded in calculating mean.

§ Two determinations by the dye-dilution method were performed on each of these animals.

ments consistently or substantially greater than this were uncommon. The salt was usually administered orally in one-gram enteric-coated tablets. The added effect of bacteremia upon the maintenance dose of cortisone-salt in these animals was surprisingly minimal.

In contrast to the varying quantities of cortisone and the sodium chloride that were required for adequate maintenance, the maintenance dose of Doca for each of the two animals within this series was more constant. For one animal, the average daily requirement was 2 mg., for the other, 3 mg. On a weight basis the maintenance dose of Doca varied from 0.09 to 0.14 mg./kg. body weight/day. Supplementation of the dietary salt did not appear necessary in the two Doca-maintained animals.

The criteria which were adapted to evaluate the adequacy of the replacement therapy provided assurance that satisfactory to excellent maintenance was accomplished in both the cortisone–sodium chloride and Doca only groups. It should be mentioned that the criteria for adequacy utilized here are those previously employed.[9] The general well being, vigor, strength, and appetite of these totally adrenalectomized dogs appeared

reasonably normal throughout these experimental studies except, terminally when some of the animals were moribund as the result of a devastating bacterial endocarditis.

RESULTS

Cardiac Output Measurements in Dogs with Intact Adrenals and Iliac and Femoral Arteriovenous Fistulas. Sixteen dogs with intact adrenals and bilateral hind extremity arteriovenous fistulas underwent cardiac output determinations employing the dye-dilution method. The results were gratifyingly consistent (Table 1), disclosing a mean cardiac index of 11.1 liters/minute with a standard deviation of 1.71 liters/minute. Thirteen of these animals had additionally a nearly simultaneous determination of their cardiac output by the direct Fick method. By this latter method, which produced much wider variations, the mean cardiac index was 16.4 liters/minute with a standard deviation of 4 65 liters/minute (Table 1). In 10 of the 13 animals in which both methods were employed, the values for the cardiac index obtained by the direct Fick method were of greater magnitude than those derived by the dye method.

Effects of Total Adrenalectomy upon Cardiac Output in Dogs with Iliac and Femoral Arteriovenous Fistulas. Seven totally adrenalectomized dogs with bilateral arteriovenous shunts had cardiac output studies, and in six of these both the dye-dilution and the Fick methods were carried out. An analysis of the cardiac indices in these animals obtained before and after bilateral adrenalectomy is presented in Table 2

In the pre-adrenalectomy studies upon these seven dogs with bilateral arteriovenous fistulas, the results of the measurements of cardiac output by the direct Fick method were with but one exception (dog No. 332) substantially higher than those obtained by the dye-dilution method. The mean difference in the cardiac index for these 7 dogs obtained with the two techniques was 5 4 liters/minute.

Following bilateral adrenalectomy the measurements obtained by the dye method, which appeared to be the technique providing the more reproducible results, demonstrated a fall in the cardiac index in 5 of the 7 animals. The reduction in these 5 animals varied in magnitude from 24 per cent to 55 per cent with a mean of 39 per cent less than that obtained with the adrenals intact

In two of the adrenalectomized animals (dogs 289 and 1106, Table 2A) the cardiac index as measured by the dye method was elevated over that measured in the pre-adrenalectomy state. In both of these animals, measurements by the Fick method also showed an elevation in the cardiac index following adrenalectomy. It should also be noted that neither of these animals had a bacteremia at the time of their postadrenalectomy cardiac output studies.

In the postadrenalectomy state the values derived by the direct Fick method were in all cases higher and in all but one instance (dog No. 289) were 100 per cent higher than those obtained by the dye method in the same animal at the same time. The mean difference obtained by the two methods in the postadrenalectomy studies was 7.8 liters. The cardiac index as measured by both techniques declined in only 2 of the 7 animals (dogs 1749 and 592) in which total adrenalectomy was carried out.

Influence of Bacteremia. In the pre-adrenalectomy state all sixteeen of the animals with bilateral arteriovenous fistulas studied had a sterile blood cul-

Table 2. Measurement of the Cardiac Index before and after Total Adrenalectomy in Dogs with Bilateral Iliac and Femoral Arteriovenous Fistulas*

DOG NO	CARDIAC INDEX PRE-ADRENALECTOMY		INTERVAL BETWEEN ADRENALECTOMY AND POSTADRENALECTOMY CARDIAC OUTPUT STUDY	CARDIAC INDEX POSTADRENALECTOMY		CHANGE IN CARDIAC INDEX POSTADRENALECTOMY WITH	
	FICK METHOD	DYE METHOD		FICK METHOD	DYE METHOD	FICK METHOD	DYE METHOD
	A. POSTADRENALECTOMY MAINTENANCE ON CORTISONE AND SUPPLEMENTARY NACL						
277‡	18.2	15.5	81 Days	21.7	11.8	19% ↑	24% ↓
289‡	16.6	10.0	23 Days	27.2	21.0	61% ↑	110% ↑
1106‡	16.2	9.8	23 Days	22.8	10.2	41% ↑	4% ↑
1201†	Not Done	13.0	60 Days	Not Done	6.0		54% ↓
1749†	13.0	10.4	61 Days	9.2	4.7	30% ↓	54% ↓
	B. POSTADRENALECTOMY MAINTENANCE ON DOCA ONLY						
332‡	9.2	9.5	21 Days	14.9	7.1	62% ↑	25% ↓
592‡	24.5	10.6	22 Days	12.3	6.6	50% ↓	38% ↓

* Cardiac Index = Cardiac output in liters/M^2 body surface/minute.
† Bacteremia present only at time of postadrenalectomy cardiac output determination
‡ Bacteremia absent during both pre- and postadrenalectomy cardiac output determination

ture at the time of their cardiac output measurements. Of the seven animals of this group available for postadrenalectomy cardiac output studies, four had sterile blood streams and three had a bacteremia at the time of their postadrenalectomy cardiac output measurements (Table 2). Of the four animals with sterile blood streams at the time of their postadrenalectomy cardiac output determinations, two were maintained on cortisone and sodium chloride and both of these animals (dogs 289 and 1106, Table 2A) showed a rise in their cardiac output by both methods of measurement following adrenalectomy. In the other two dogs without bacteremia but maintained upon Doca only (dogs 332 and 592, Table 2B) the cardiac indices measured by the dye method fell in both animals, but in only one of these two dogs when measured by the Fick method. Of the three dogs with bacteremia at the time of their postadrenalectomy measurements (dogs 277, 1201, and 1749, Table 2A) only two had volume output measurements by both methods and in only one of these (No. 1749) was the cardiac output reduced by both methods following adrenalectomy. In the other (dog No. 277), the dye method showed a reduction and the Fick an increase in the cardiac index.

DISCUSSION

The observation that dogs with s [illegible] protected against cardiac valvular l [illegible] tomized and maintained upon Doc [illegible] coristone and sodium chloride were as susceptible to bacterial endocarditis as normal dogs with arteriovenous shunts, appeared to afford an excellent opportunity for investigating basic mechanisms associated with resistance and susceptibility to bacterial infecton especially as it pertains to the heart valves.

At least two plausible explanations for these observed differences seemed possible. The first invoked a purely "mechanical" hypothesis based upon the possibility that extirpation of the adrenal glands followed by maintenance upon Doca alone substantially reduced the increased work load of the heart associated with the large arteriovenous shunts, and that the contrary occurred when the adrenalectomized animals were maintained upon cortisone. The frequent association of a state of increased endocardial vulnerability to infection and conditions which invoke protracted increases in cardiac work has been emphasized previously by both experimental and clinical observations.[1, 10]

Another possible hypothesis was that, as numerous reports have suggested, cortisone had a direct inhibitory effect upon the body's natural defense mechanisms as well as possibly a specific effect enhancing localization of the bacterial growth in the heart valves, these effects being independent of any alterations in the mechanical or physical work of the heart. These studies have provided several observations pertinent to these problems.

First, it was quite apparent that the dogs with large vascular shunts and adrenalectomy maintained upon cortisone and sodium chloride were easier to manage and were more nearly normal in their vigor, appetite, and appearance than the Doca-maintained dogs, although it is emphasized that the latter dogs were outwardly not sick. This observation was further borne out by the fact that all of the cortisone-treated dogs survived the added stress of anesthesia and their cardiac output determinations, whereas the mortality

was 100 per cent in the Doca-maintained animals in spite of vigorous supportive therapy.

It is apparent from this study that there were rather striking variations in the volume output determinations when the two methods were used nearly simultaneously in measuring the cardiac indices in dogs with bilateral arteriovenous fistulae (Tables 1 and 2), Thus, conclusions based upon the data derived from these cardiac output measurements by the two methods employed are to some extent depreciated by the variability observed

These two methods, the direct Fick and the dye-dilution technique, have been compared previously under normal conditions by performing the two nearly simultaneously both in dogs[9] and in man [11] These studies were in general agreement, indicating that the results were quite comparable in the normal or steady state and that the errors of one method were no greater than those of the other under these study conditions, nor was there any apparent systematic error. However, more recently Visscher and Johnson[12] have emphasized certain possible errors inherent in the application of the Fick principle when the subject is not in an absolutely steady state. Further, as an example of this variation, Nahas, Haddy, and Visscher[13] demonstrated that although the Fick and dye-dilution measurements of cardiac output in the same animal agreed well when their dogs were in a normal state the presence of hypoxia caused a substantial and systematic difference between nearly simultaneous determinations in the same animal, owing, apparently, to the errors inherent in the methods in the absence of such a steady state.

As indicated above, wide differences in the cardiac index were observed in our animals with vascular shunts both with intact adrenals and in the same animals when the cardiac output was measured at varying intervals after total adrenalectomy. In the pre-adrenalectomy cardiac output studies the mean cardiac index determined by the direct Fick procedure was 47 per cent higher for the same animal than that calculated utilizing the dye-injection method (Table 2) In the postadrenalectomy studies the mean cardiac index determined by the direct Fick procedure was 85 per cent greater than that derived almost simultaneously by the dye-injection method (Table 2). Thus, in a perponderant number of determinations the volume outputs obtained by the direct Fick procedure were substantially greater than those determined by the Hamilton method

The basis for this apparent systematic error is not clear but may well be related to the presence of the arteriovenous shunts. The relatively small deviations in the values obtained by the dye-injection method before adrenalectomy (Table 1) were gratifying, but there was a considerably wider divergence in the results obtained by the same method following total adrenalectomy (Table 2 A, B) This latter observation likewise may reflect the greatly altered physiologic state of these animals. The results of both the dye and Fick methods confirm the marked elevation of the cardiac indices observed previously in dogs with arteriovenous fistulas and intact adrenals.[14]

With due allowance for these discrepancies, the cardiac output determinations in this study do tend to offer some support for the hypothesis that alterations in the susceptibility of the heart to infection may in part at least be explicable on a purely mechanical basis It may be noted that the

effect of total adrenalectomy with cortisone maintenance, in the two dogs in which a deliberately induced bacteremia was not present to complicate the measurements, resulted in an increased cardiac output above the pre-adrenalectomy levels (as measured by both methods) (Table 2 A, dogs 289 and 1106). As previously noted, it is in these cortisone-maintained adrenalectomized animals that the heart valves remained susceptible to bacterial infection, whereas in the Doca-maintained group, which were not susceptible to valvular carditis, the cardiac index following adrenalectomy appeared to be substantially reduced in three out of four of the determinations (Table 2 B).

SUMMARY

Nearly simultaneous determinations of the cardiac output were made by the direct Fick and the dye-dilution methods in dogs with bilateral iliac and femoral arteriovenous fistulas before and after total adrenalectomy, with some of the animals maintained after adrenalectomy upon Doca only and the others being maintained upon cortisone with supplemental sodium chloride.

In the 16 dogs with bilateral arteriovenous shunts and intact adrenals the cardiac index as measured by dye-dilution method was 11.1 ± 1.71 liters/minute and measured by the direct Fick procedure in the same dogs was 16.4 ± 4.65 liters/minute.

Following total adrenalectomy the measurements of cardiac output by both methods tended to be more variable, and the direct Fick method gave consistently higher values than those obtained by the dye-dilution method in the same dogs.

Total adrenalectomy in the dogs with bilateral vascular shunts, sterile blood streams, and maintenance upon a cortisone–sodium chloride regimen resulted in a further increase in the cardiac output over that observed in the pre-adrenalectomy state by both methods of measurement. Total adrenalectomy in similar animals, except that maintenance was by Doca alone, caused a reduction in the cardiac output

These observed effects of total adrenalectomy upon cardiac output as influenced by the character of the maintenance therapy may offer an explanation of the previously observed differences in susceptibility to valvular bacterial endocarditis associated with altered adrenal function in dogs with sustained arteriovenous shunts

Under the experimental conditions of sustained cardiovascular stress, significant systematic differences in the cardiac output in the same animals as measured by the direct Fick and the dye-dilution methods were demonstrated.

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the magnitude and duration of the increased work thrust upon the heart.[4] Moreover, endocrine factors have been found to play an important role as illustrated by the alterations in susceptibility associated with adrenalectomy.[5] Since the thyroid has both an indirect mechanical[6, 7] and possibly a direct metabolic effect upon the heart,[8] the following studies have been undertaken to determine the influence of changes in thyroid function upon the cardiac susceptibility to bacterial infection.

METHODS

The twenty-eight animals used in these studies were medium-sized adult mongrel dogs of either sex, previously dewormed, immunized against distemper and acclimated to the animal colony before operation. They were fed daily a diet consisting of dog biscuits supplemented with fresh horse meat and milk.

Arteriovenous Fistulae. These were constructed as previously described.[2] They measured between 20 and 25 mm. in length and were made side to side between the iliac and femoral arteries and veins. Since their length was made more than twice the diameter of the parent vessels, possible effects due to variations in the size of the different animals were minimized

Bacteriology A course of bacterial injections was instituted between three and six weeks after construction of the shunts. It consisted of 1 cc. of a 24 hour culture of strain A_{12} beta-hemolytic streptococci grown in brain heart infusion broth and given intravenously each day for seven days. Colony counts on this material averaged 4×10^{9}/cc. of broth. Following the completion of the bacterial injections, the clinical course of the dogs was followed by twice weekly auscultation of the heart and blood cultures, and with daily measurements of their body weight, rectal temperature and hemoglobin. All animals were autopsied at the time of death.

Group I [illegible] s acted as controls for the observation [illegible] oup III (hypothyroid) animals.

[illegible] *Fistulae.* Nine dogs had an iliac arteriovenous fistula constructed on one side and a femoral arteriovenous fistula on the other leg. Following the recovery period they received the standard course of bacterial injections

With Single Iliac Arteriovenous Fistula. To determine whether alterations in thyroid function made dogs more susceptible to endocarditis, animals were prepared with a single iliac arteriovenous fistula. Four such euthyroid dogs received the standard course of injections after a three to six week recovery period

With Single Femoral Arteriovenous Fistula. Similarly two dogs with single femoral fistulae were injected to act as controls and to confirm previous observations that such animals do not develop bacterial endocarditis.[2]

Group II: Hyperthyroid Dogs. Desiccated thyroid extract U S.P. was administered orally to four animals. Because of the experiences of Danowski et al[9] demonstrating the remarkable resistance of euthyroid dogs to this medication, a high dose of 1 0 grams per day was given. Basal metabolic rates were determined under anesthesia by the method of Bartels[10] before and at intervals after the exhibition of the drug. Hyperthyroidism, as determined by measured elevations in oxygen consumption, was observed to develop three to four weeks after the start of the administration of the thyroid extract. At this time, single femoral arteriovenous fistulae were pro-

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THE PRODUCTION OF HYPO- AND HYPERTHYROIDISM IN THE DOG*

Preliminary Observations Regarding the Effect of These States on the Susceptibility of Dogs with Arteriovenous Fistulae to Bacterial Endocarditis

RAYMOND C READ, JAMES F. MARVIN, AND C. WALTON LILLEHEI

In 1950, it was observed that dogs with large arteriovenous fistulae frequently died after some weeks of "spontaneous" bacterial endocarditis whereas animals with smaller shunts were unaffected[1] Subsequent studies showed that the dogs with the larger arteriovenous shunts were remarkably susceptible to valvular infection following the intravenous injection of microorganisms in numbers innocuous to normal animals[2] As a result of these observations a new method for the uniform production of bacterial endocarditis was developed[3] Utilization of this experimental preparation has made it possible to analyze under controlled conditions some of the factors involved in the susceptibility of the heart to infection. It has been demonstrated that the observed increase in susceptibility is proportional to

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O_2/kg. body weight. Similar tests obtained 3 weeks later after the production of single femoral fistulae and at the start of the bacterial injections gave a mean of 26 cc. O_2/kg. with a range of 23 to 31 cc. O_2/kg. body weight. All of these animals lost weight and became irritable and hyperactive while receiving thyroid in these amounts. However, when the three afebrile dogs were re-examined after a further two weeks, which was 9 weeks after the start of the hormone, the O_2 consumption had returned to a normal range 11.1 to 13.6 cc. O_2/kg. body weight (Fig. 1).

Single Femoral Fistulae in Hyperthyroid Dogs. Three of these four animals rapidly cleared their blood of bacteria following the course of injections and remained well. The other dog developed a persistent septicemia over a period of 8 weeks. However, he finally sterilized his blood stream and remains alive and well.

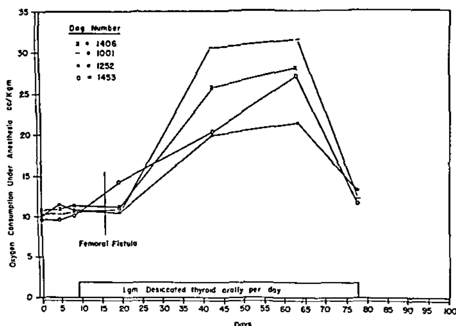


Fig 1 Effect of desiccated thyroid on the oxygen consumption of four dogs under anesthesia.

Group III: Hypothyroid Dogs. Two animals died of distemper before completing their radioiodine therapy. They received 1 mc./kg. and 4 mc./kg. of radioiodine respectively, and both showed functioning thyroid by tracer uptake and histologic studies. The next 3 dogs also still showed thyroid activity by tracer studies after receiving total doses of 4 mc./kg. However, following a further 3 mc /kg. of body weight no remaining thyroidal function could be demonstrated by external counting. The last 4 dogs received total doses of 6 mc./kg. and were similarly athyroid. The average uptake of I^{131} by the thyroid glands of these dogs following tracer doses at 24 hours was 5 per cent; later therapy doses showed 10 per cent uptake at 3 days. The isotope disappeared initially at a rapid rate but later the rate of loss of radioactivity from the thyroid slowed considerably. Its initial half-life was one day and later between 3 and 5 days.

Estimation of the Roentgen Dose Required to Ablate Thyroid. Six mc./kg. was found to be sufficient to ablate the thyroid, whereas 4 mc./kg. was

duced in these animals After the usual three week recovery period during which the administration of thyroid extract was continued they were injected with bacteria. The thyroid medication was then continued further until the death or sacrifice of the animal.

Group III: Hypothyroid Dogs. Radioactive iodine was given to nine dogs.* The activity of the thyroid was destroyed in seven animals, the remaining two dying of distemper before completing their treatment The carrier free potassium iodide (I^{131}) was injected intravenously in doses of between 1 and 3 mc./kg. body weight at intervals of 3 to 4 weeks. The percentage uptake by the thyroid was measured the day after injection with a Geiger counter over the neck. The rate of disappearance of the radioiodine from the gland was followed by the same technique. An average weight per kilogram body weight for the dogs' thyroid was obtained from autopsy studies, making it possible to calculate the gram-roentgen dose to thyroidal tissue. Tracer doses of KI^{131} (100 to 200 μc.) were used to measure the function remaining after each treatment. The isotope was continued until no further uptake could be elicited and the counts over the neck became essentially the same as those over the rump In four animals oxygen consumption measurements were made before and after the development of myxedema. Three grades of arteriovenous fistulae (i.e., iliac and femoral, single iliac and single femoral) were produced in these athyroid dogs and they were injected with bacteria as previously outlined

RESULTS

Group I: Euthyroid Dogs. *Bilateral (Iliac and Femoral) Arteriovenous Fistulae*

The nine dogs in this series developed fever, septicemia, anemia, and cachexia following the standard course of streptococcal injections. All died between 4 and 30 days after receiving the bacteria. At autopsy valvular vegetations, petechiae and other evidences of endocarditis were found In one dog the final cause of death was a ruptured mycotic aneurysm at the site of the iliac fistula.

Single Iliac Arteriovenous Fistulae Four dogs with single iliac fistulae showed exactly the same response to the standard injections of streptococci. All died within one month of bacterial endocarditis with a continuous septicemia

Single Femoral Arteriovenous Fistulae Two dogs with single femoral fistulae cleared their blood of the injected bacteria and are still living in good health at the present time with no evidence of blood stream infection

Group II: Hyperthyroid Dogs. The reproducibility of the oxygen consumption measurements was examined by performing triplicate determinations at intervals over a period of one month in four normal dogs of similar weight Great care was taken to keep the anesthetic conditions (Pentothal with endotracheal oxygen) as constant as possible The mean value obtained for the oxygen consumption in these dogs in their normal state was 10 6 cc. O_2/kg. body weight with a range for the 36 determinations of 9 to 14 cc. O_2/kg. of body weight These studies were repeated after the dogs had been on thyroid for one month The average oxygen consumption for these same animals was then 25 cc. O_2/kg with a range of 21 and 31 cc.

* Supplied through the Atomic Energy Commission, Oak Ridge, Tennessee

carditis 4 to 36 days after their last injections. These results are similar to those observed in the euthyroid dogs with the same size shunt.

Single Iliac Fistulae. In two hypothyroid dogs single iliac fistulae were created They were resistant to bacteria as manifested by continuing good health.

Single Femoral Fistulae. Two of these athyroid animals had single femoral fistulae produced. Each died with endocarditis but complicating conditions were present. One developed distemper and the other, instead of having had previously a simple thoracotomy under hypothermia, was found at autopsy to have had as well an iatrogenic mitral insufficiency.

Autopsy studies on the dogs who died confirmed the complete destruction of the thyroid. This structure was reduced to a fibrous strand surrounding normal parathyroid tissue. No viable thyroid tissue could be found in the histologic sections (Fig. 2).

DISCUSSION

The uniform incidence of bacterial endocarditis in the nine euthyroid control animals with bilateral arteriovenous fistulae is a further confirmation of the reproducibility of this method for the experimental production of subacute bacterial endocarditis The susceptibility of dogs with single iliac fistulae to this lesion indicates that even a lesser cardiovascular stress is sufficient to lower the vascular resistance to infection with the strain and number of organisms used in this study. The observed resistance of the single femoral fistula dog to endocarditis also confirms previous work[3] and illustrates that the range between femoral and iliac fistulae is where decreased resistance of the heart to infection first becomes manifest under these experimental conditions.

The experiences with the experimental production of hyperthyroidism confirm Danowski's work showing that it is possible to produce this state [illegible] g [illegible] l.

This temporary hyperthyroid state did not increase the susceptibility of single femoral fistula dogs sufficiently to make them susceptible to valvular endocarditis. In one dog with a single femoral arteriovenous fistula a severe septicemia developed, but this animal gradually cleared his blood stream over a period of 8 to 10 weeks. This case is perhaps of some significance because of the uniform absence of septicemia in single femoral arteriovenous fistula dogs found in a number of previous experiments as well as in the euthyroid controls in this study It may be postulated this dog might have died and the resistance of the others become more profoundly decreased if the hyperthyroid state could have been maintained.

The production of hypothyroidism in the dog with radioiodine confirms the work of Chaikoff[11] This method avoids damaging the parathyroids (impossible to do otherwise in the carnivora with surgery—Marine[12]) and also completely destroys all aberrant thyroid tissue. It is, however, time consuming The dosage required to destroy measurable thyroid function, three separate 2 mc./kg. at three weekly intervals, is greater than that used by Chaikoff who, however, found thyroid persisting in two out of eight animals. The clinical myxedema produced is also contrary to the results of earlier workers using different techniques[13-14] but is substantiated by

not enough. The average weight of the thyroid was 0.02 per cent body weight (6 dogs). Mean uptake of therapy doses at 3 days was 10 per cent, therefore, concentration of isotope in thyroid gland was 3 mc./gram of thyroid This tissue dose is in the same range as that used to completely ablate the thyroid in the human (0 25 to 1 mc. gram) where the half-life in the gland may be as long as six days compared to the 1 to 3 day half-life



Fig 2 Ph [illegible] , of thyroid and parathyroid *Above* shows slight effect with 4 [illegible] ., destruction of the thyroid glandular tissue with 6 mc of radioiodine/kg body weight

found in these animals. Four of these seven animals were clinically myxedematous. They gained weight, became sluggish and developed thick jowls and wrinkled skin. These animals when tested at 3 months after exhibition of the radioiodine had reduced their oxygen consumption by between 20 per cent and 30 per cent. The remaining 3 did not show changes clinically

Bilateral Arteriovenous Fistulae. In four of these hypothyroid animals both iliac and femoral arteriovenous fistulae were produced. They responded uniformly to the streptococcal injections dying with bacterial endo-

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ELECTROCARDIOGRAPHIC CHANGES FOLLOWING BILIARY AND GASTRIC DISTENTION IN FRESHLY INFARCTED UNANESTHETIZED DOGS*

RICHARD L. WECHSLER, S. BELLET, A. K. KAPLAN, AND PAUL NEMIR, JR.

The possibility that cardiac changes may be caused by pathologic or physiologic abnormalities in the abdominal organs has important clinical implications.

The effect of reflexes from the gastro-intestinal tract upon the heart has been the subject of many reports in the literature.[1-3] Much of the work was done in the experimental animal with varying degrees of anesthesia.[4, 6, 7] In addition, some data are available regarding the effect on the heart of distention of the colon, stomach, gallbladder and bile ducts in the human subject under anesthesia [2, 3, 5] The observation in human subjects that abdominal distention produced little or no objective cardiac effect in the normal subject, whereas a similar event in a patient with coronary artery disease often reproduced pain and transiently altered the electrocardiogram, stimulated us to do the studies described below. The mechanism by

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the oxygen consumption studies showing a substantial fall in the O_2 consumption of these animals. Again it is found that apparently species makes a difference in the response to thyroidectomy, as only about half of the animals did show clinical myxedema. The susceptibility of these few hypothyroid animals to bacteria was not significantly different from that of normal dogs. However, in two animals with a single iliac arteriovenous fistula in which the increased cardiovascular work, and thus presumably the susceptibility to infection, was less than the bilateral fistulae (though still enough to induce endocarditis in the euthyroid dog subjected to bacteria), hypothyroidism did appear to offer some protection against the occurrence of bacterial endocarditis. Unfortunately this suggestion remains equivocal owing to the fact that both of the hypothyroid animals with single femoral arteriovenous fistulae died of bacterial endocarditis. However, as mentioned above, other complicating factors were present in these animals which may conceivably have decreased their resistance to infection.

All of these animals received a high iodide intake in their standard dog biscuits (1 per cent iodized salt). They also received meat. Both of these sources may lead to thyroxine-like activity.[11] By a specifically deficient diet the iodine pool in the body may be markedly reduced and thus it may be possible to increase the degree of myxedema and simultaneously decrease the amount of radioiodine needed to ablate the thyroid.

SUMMARY

Earlier observations upon the susceptibility to bacterial endocarditis of dogs with bilateral fistulae and their resistance when only a single femoral arteriovenous shunt is present have been confirmed. The present study indicates that euthyroid dogs with single iliac fistulae are also uniformly susceptible to cardiac infection by this method. A temporary hyperthyroid state induced with massive doses of thyroid hormone has been produced in four dogs with single femoral arteriovenous fistulae. All four of these dogs failed to develop bacterial endocarditis. However, one animal showed a persistent septicemia from which a spontaneous recovery occurred over a period of 10 weeks. Myxedema, as demonstrated by oxygen consumption, tracer uptake and histologic studies, was produced with I^{131} in seven dogs. This state did not alter the susceptibility of animals with bilateral arteriovenous fistulae to endocarditis but did appear to protect two dogs with single iliac shunts against the development of vegetative endocarditis.

CONCLUSION

Alterations of thyroid function within the limits of this study gave suggestive but inconclusive evidence of changes in the susceptibility of the cardiac valves to bilateral endocarditis

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infarction showed occasional extrasystoles, gastric distention resulted in a marked increase in the number of ectopic beats or in a ventricular tachycardia. In two instances the maintenance of inflation for 10 minutes resulted in the development of a slow idioventricular rhythm with respiratory arrest. In one of these cases immediate deflation resulted in a return to the control tracing. The results of biliary distention were similar to those observed after

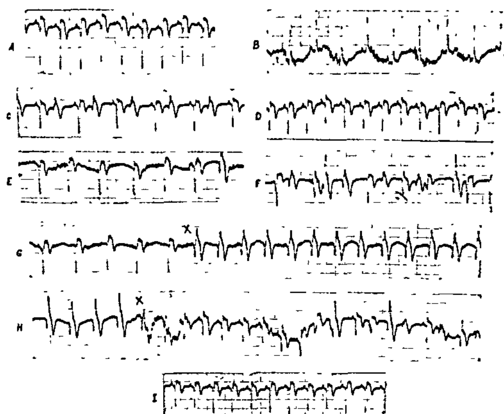


FIG 1 Stomach distention, 24 hours after coronary ligation *A* (V4), Control tracing Note absence of R wave and elevatic[illegible] infarction *B* (L2), Beginning dist[illegible] base line and somatic tremor *C* (V4[illegible] of ectopic beats which are coupled[illegible] to normal rhythm *E* (V4), Beginn[illegible] sional ectopic beat at end of tracin[illegible] frequent multifocal ectopic [illegible] tion of ectopic tachycardia, [illegible] tinued distention of stomach [illegible] beats *I* (V4), Released dis[illegible] ectopic beats

gastric distention (see Fig. 2). However, these changes were more difficult to produce and less frequent during biliary distention.

After the fifth or sixth postoperative day, the effects of biliary and gastric distention noted above tended to decrease gradually and were usually not observed after the sixth to eighth postoperative day. The S-T segment changes resulting from the distention were inconspicuous.

Quinidine, Pronestyl and atropine partially protected the animals with myocardial infarction from the development of the ectopic rhythms due to

which these procedures cause the symptoms and electrocardiographic changes is unknown We hoped to help clarify this mechanism.

METHODS

Gastric or biliary distention was induced in six normal anesthetized (Amytal) dogs, six normal unanesthetized dogs, twelve gastric distention and six biliary distention dogs in the acute, subacute and healed stages of myocardial infarction produced by ligation of the anterior descending branch of the left coronary artery. In addition, in five of the unanesthetized animals having myocardial infarction, intramuscular atropine (two dogs), quinidine (one dog), procaine amide (one dog) and acetylcholine (one dog) were given Mean arterial and venous blood pressures were recorded by means of a strain gauge manometer in two dogs.

Gastric distention was produced by the insertion of a balloon through a gastrostomy opening The balloon was distended slowly with 600 to 1400 cc. of air depending on the size of the animal, and this distention was maintained for 3 to 10 minutes and then gradually released. Biliary distention was produced by means of a balloon-tipped catheter which was inserted through the common duct into the gallbladder at a previous operation. By the use of a T tube connected to the catheter and a saline manometer, distention of the gallbladder and biliary tree was maintained for 20 minutes at a pressure of 1000 mm of normal saline Frequent electrocardiograms were taken before, during and following the distention Although limb leads were studied, the precordial leads V3 and V4 were used routinely in these experiments.

RESULTS

Distention of the stomach or biliary tree in the normal anesthetized or unanesthetized dogs failed to produce significant electrocardiographic changes except for a mild tachycardia or vagal arrhythmia. There were no significant R-T or T wave changes or ectopic rhythms.

Myocardial infarction produced by ligation resulted in the typical QRS and S-T segment changes Extrasystoles and short paroxysms of tachycardia are frequently observed within 24 to 48 hours after operation They usually disappear at the end of this time

After myocardial infarction the electrocardiographic changes produced by gastric or biliary distention of the anesthetized dog were not significant except for an occasional extrasystole, tachycardia or occasionally a slight increase in R-T segment elevation

If the control electrocardiogram showed a regular sinus rhythm two to six days after acute infarction, gastric distention in five of six unanesthetized animals resulted in the development of multiple extrasystoles, runs of ventricular tachycardia, coupled rhythm, and sinus arrhythmia (Fig. 1). The R-T segment deviation tended to be more marked and the T wave inversion greater during the period of distention and for 5 to 10 minutes after release. The electrocardiogram reverted to the control tracing after this period. These observations were repeated 2 to 4 times during the period of one hour. With each successive trial on the same day, the ectopic rhythms were less pronounced At times, the ectopic rhythm was absent during the period of inflation but appeared during the period of deflation. Where the control electrocardiogram in the first 24 to 48 hours following

distention is not known. Vagal reflexes from the gastro-intestinal tract and gallbladder are known to occur and have been implicated as the mechanism. Atropine, the peripheral parasympathetic blocking agent, did decrease the number of ectopic rhythms. However, it did not abolish them completely, and atropine may have other direct cardiac or central nervous system actions which could cause the observed changes. Anoxia may occur as a result of the distentions of the abdominal organs. This anoxia could cause the arrhythmias and R-T segment and T wave changes. A third possible mechanism is an increased secretion of epinephrine due to the pain produced by biliary and gastric distention. The fact that the unanesthetized dogs showed much evidence of pain and that the electrocardiographic changes did not occur when pain was prevented by anesthesia is suggestive but not conclusive evidence for this mechanism. The results with quinidine and Pronestyl indicate that the arrhythmias may be due to an abnormality of the myocardium.

SUMMARY

1. The effect of gastric and biliary distention was studied on the electrocardiograms of normal dogs with and without anesthesia and at various periods following acute infarction produced by coronary artery ligation.

2 Gastric and biliary distention produced little or no significant changes in the anesthetized animals and in the unanesthetized normal animals.

3. In the acute stage of myocardial infarction, two to five days past infarction, gastric and biliary distention resulted in the production of multiple extrasystoles, ventricular tachycardia coupled rhythm and sinus arrhythmia. In addition, elevations of the R-T segment and T wave inversions were observed in the period immediately following distention. These changes were more evident following gastric than biliary distention.

4. In the doses given, quinidine and Pronestyl partially protected the animals from the development of ectopic rhythms following distention.

5 The clinical implications and possible mechanism of these results are discussed.

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gastric distention. There were only occasional extrasystoles and runs of ventricular tachycardia following the use of these drugs. Acetylcholine increased the incidence of cardiac arrhythmias. The mean arterial and venous blood pressures rose during gastric distention in two animals. These pressures returned to normal upon release of the distention.

Discussion The fact that in these dogs we were unable to produce electrocardiographic changes by distention of the stomach or biliary tree unless the animals had fresh myocardial infarcts, reaffirms the previously suggested

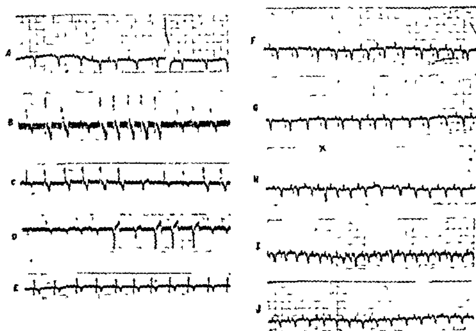


FIG 2 Gallbladder distention, 48 hours (*A-E*) and 72 hours (*F-J*) after coronary ligation *A* (L2), Control tracing *B* (L2), Gallbladder distention (immediate). Note numerous ventricular extrasystoles and a sequence of 5 *C* (L2), Gallbladder distention, 5 minutes post distention Note numerous ectopic beats *D* (L2), Gallbladder distention, 10 minutes post distention Note frequent ectopic beats *E* (L2), Release of distention and aspiration of gallbladder, 5 minutes post release Note return to a normal rhythm *F* (L2), Control tracing, normal rhythm *G* (L2), Gallbladder distention, immediate No ectopic beats *H* (L2), Gallbladder distention, 5 minutes post distention Occasional ectopic beats are observed at *X* *I* (L2), Gallbladder distention, 10 minutes post distention Normal rhythm *J* (L2), Release of distention and aspiration of gallbladder, 5 minutes post release Normal rhythm

idea[3,4,8] that some degree of cardiac disease is necessary in order for abnormalities of the abdominal organs to produce cardiac changes It also adds more experimental evidence to the concept that cardiac signs and symptoms may be intensified by diseases of the abdominal organs, and therefore these organs should be studied in all cardiac cases[9] Evidence of pathology in these organs may be an indication for surgery

For five to six days after myocardial infarction the infarcted area in the dog is at its maximum extent The myocardium at this time is in a highly irritable state and is susceptible to the effect of various stimuli. The mechanism of the electrocardiographic changes following gastric and biliary

BLOOD VESSELS AND CIRCULATION

OBSERVATIONS ON THE VENOUS CIRCULATION TIME IN THE LOWER EXTREMITIES: EFFECT OF ELEVATION AND COMPRESSION BANDAGES*

PAUL F. PAULSEN, OSCAR CREECH, JR., AND MICHAEL E. DE BAKEY

It has long been recognized that posture has a significant effect on the venous return to the heart from the lower extremities. Piorry[12] in 1826 noted that subjects kept in the upright position developed syncope that was relieved by recumbency. This he attributed to the effect of gravity on the venous blood flow. In animal experiments Hall[4] observed that the venous return to the heart was largely dependent on position and that gravity was a controlling factor in the distribution of blood to the lower limbs. The implication, by Virchow,[16] of venous stasis as one of the etiologic factors in venous thrombosis stimulated further interest in the effects of posture on the venous blood flow in the lower extremities. In 1895 Hill[6] noted that an increased rate of venous flow occurred when the body was tipped head downward, and more recently other investigators[17, 18] have confirmed these findings. Kvale and associates,[8] and Smith, Allen and Craig,[14] have observed an 8 to 25 per cent decrease in the foot-to-carotid circulation time when the leg is elevated from the horizontal position. Clinically, elevation of the lower limbs above heart level has been employed to reduce the incidence of venous thrombosis.[11] Similarly, compression bandages have been wrapped about the lower extremities of postoperative patients in an attempt to prevent venous thrombosis.[2, 9, 10] The effect of this latter procedure on the venous circulation time, however, has not been demonstrated by measurements of the venous flow rate in the lower limbs

This study was undertaken to determine the effect (1) of elevation, (2) of application of elastic bandages, and (3) of these two measures combined on venous circulation time in the lower extremities.

METHODS

The technique for measuring the venous circulation time in the lower extremities has been described elsewhere[1] and is essentially as follows. Subjects are kept recumbent with the legs covered by a blanket for 15 minutes prior to the tests. A shielded scintillation counter with an aperture ⅝ inch by 2 inches is placed over and perpendicular to the femoral vein at the inguinal ligament. The internal saphenous vein at the ankle is selected as the site for injection After application of a tourniquet a 21 gauge 1½ inch needle connected to a 2 cc. syringe containing saline is inserted into the vein. The tourniquet is released and a small quantity of saline is injected slowly to make certain the needle is properly placed The syringe containing saline is replaced with one containing 0 75 cc. (30 microcuries) of human serum

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the toes to just below the knee. The second injection of the isotope solution was then made and the circulation time determined.

To compare the effects of elevation alone, and of elevation with application of an elastic bandage to the extremity on the venous circulation time in the lower limbs, 100 subjects were studied. Their ages ranged from 18 to 60 years, and they were all ambulant and without vascular disorders.

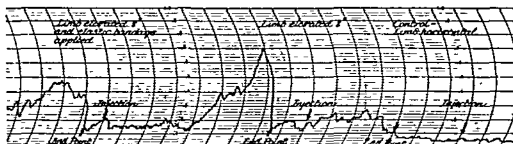


Fig. 2 Photograph of the record obtained during determination of venous circulation time in the lower extremity (1) with the limb horizontal, (2) with the limb elevated eight inches, (3) after application of an elastic bandage to the elevated limb.

Three separate determinations were made as follows: (1) with the limb horizontal and the site of injection at the phlebostatic level; (2) with the limb elevated and the site of injection eight inches above the phlebostatic level, the foot being supported at the heel; (3) with the limb elevated, the site of injection being eight inches above the phlebostatic level, and an elastic bandage applied from the base of the toes to the tibial tubercle (Fig. 2). The time elapsing between each determination varied from 5 to 20 minutes, depending upon the rapidity with which the counting rate returned to a background level.

RESULTS

In eight of the ten subjects initially studied to determine the effect of elevation on the venous circulation time, a significant decrease occurred after elevation, and in two cases an increase was noted (Fig. 3). Of 20

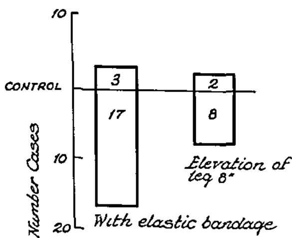


Fig 3. Venous circulation time in lower extremities, experimental studies.

albumin labeled with I^{131} The scintillation counter is connected to a milliammeter recorder that has been calibrated with reference to rate of motion. When sufficient background has been obtained the radioalbumin is injected as rapidly as possible. Approximately 1.5 seconds are required for injection; the start of injection marks the beginning of the record. Arrival of the albumin at the groin is indicated by a sudden increase in counting rate. The time from start of injection to appearance of the isotope at the groin represents the venous circulation time in seconds from foot to groin The straight line distance from site of injection to aperture of the scintillation counter is measured and converted to a standard of 80 cm., and the circulation time is then expressed in seconds per 80 cm.

The reproducibility of the venous circulation time by this method was tested on ten normal subjects The determinations were made under standard conditions, i e., the limb horizontal and the site of injection at phlebo-

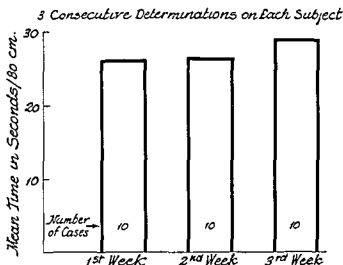


Fig 1 Venous circulation time in lower extremities, control study

static level Each subject was studied once a week for three consecutive weeks The results indicate that when repeated determinations are made on the same subject under standard conditions, the differences in circulation time are not significant (Fig 1)

The effect of elevation on the venous circulation time was investigated in ten subjects. The tests were performed first with the leg horizontal and the site of injection at phlebostatic level When the end point was reached and the counting rate had returned to background level, the leg was elevated so that the site of injection was eight inches above the phlebostatic level, and the second injection of the isotope solution was made.

The effect of elastic compression bandages on the circulation time in the lower extremities was determined on 20 subjects. Two determinations were made on each subject The first was performed with the leg horizontal and the site of injection at the phlebostatic level After the end point had been reached and the counting rate had returned to background level, a three-inch elastic bandage was wrapped snugly about the limb from the base of

limb elevated alone, 88 per cent of the subjects demonstrated a decrease in foot-to-groin circulation time after application of an elastic bandage, and 9 per cent showed an increase in the circulation time (Fig. 7). The effect of application of an elastic compression bandage to the elevated lower

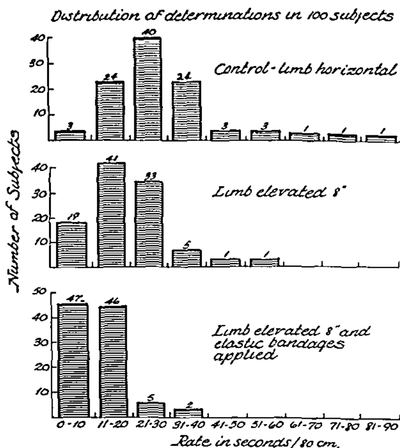


Fig 5 Venous circulation time in lower extremities, distribution of determinations in 100 subjects

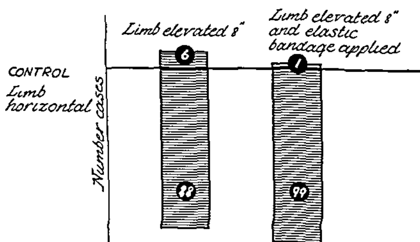


Fig. 6 Venous circulation time in lower extremities Effect of elevation alone and with application of elastic bandage.

subjects studied to determine the effect of application of an elastic bandage, 17 showed a significant decrease after application of the bandage compared to the circulation time with the limb in the horizontal position (Fig. 3). In most instances this decrease was as great as 100 per cent of the control (horizontal) determination (Fig 4).

In determining the foot-to-groin venous circulation time in 100 subjects with the limb horizontal, with the limb elevated eight inches above heart level, and after application of an elastic bandage to the elevated limb, the following results were obtained With the lower extremity in the horizontal position the shortest foot-to-groin time was 6.7 seconds and the longest 85.9 seconds, with an average of 29.1 seconds. Among the 100 determinations 40 per cent were within the range of 21 to 39 seconds (Fig. 5). In these same subjects with the lower extremity elevated and the site of injection eight inches above the phlebostatic level the shortest foot-to-groin venous circulation time was 4.8 seconds, and the longest 40.3 seconds, with an average of

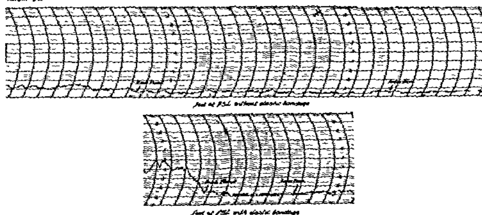


Fig 4 Photograph of record obtained during determination of the venous circulation time in the lower extremity before and after application of an elastic compression bandage

19.2 seconds. Among these determinations 74 per cent were within the range of 11 to 30 seconds (Fig 5) Nineteen per cent of the subjects had venous circulation times less than 10 seconds. After application of an elastic bandage to the elevated extremity, the foot-to-groin venous circulation time ranged from 1.4 seconds to 32 seconds, with an average of 12.4 seconds Eighty-eight per cent of the subjects had venous circulation times in the lower extremities within the range of 0 to 20 seconds (Fig. 5)

Thus in 100 subjects elevation of the lower extremity resulted in decrease in the foot-to-groin circulation time in 88 per cent of the subjects while in 6 per cent the circulation time was prolonged (Fig. 6) The effect of elevation was to decrease the venous circulation time an average of 34 per cent over the circulation time with the limb in the horizontal position

With the limb elevated and an elastic bandage applied from the toes to the knee, 99 per cent of the subjects demonstrated a decrease in the foot-to-groin circulation time compared to the determinations with the limb in the horizontal position, and in 1 per cent a prolonged circulation time was obtained (Fig. 6). When compared with the determinations made with the

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THE EFFECT OF SUBTOTAL ADRENALECTOMY UPON THE DEVELOPMENT OF ASCITES IN CHRONIC HEART FAILURE*

JOSEPH L. SPRAFKA, DONALD W. HANNON, AND IVAN D. BARONOFSKY**

The clinical management of patients with excessive fluid and salt retention, from whatever cause, frequently presents a difficult problem. Familiar to all are the difficulties encountered in the treatment of intractable ascites and peripheral edema in cirrhosis and chronic cardiac decompensation. A great deal is known about the effects of hydrostatic and intra- and extravascular colloid osmotic pressures upon the movement of fluid and electrolytes within the body. Recently interest has been focused upon the role played by various endocrine organs in the regulation of fluids and electrolytes.[2, 3] Previously we have found that the development of ascites in the experimental animal can be correlated with the elevation of right atrial pressure.[4] At this time we should like to present data on the effects of subtotal adrenalectomy upon the development of ascites in chronic heart failure.

MATERIAL AND METHODS

Chronic heart failure has been produced in dogs by creating a pulmonary

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This work was supported by Grants from th[illegible] of Health, Public Health Service, the Life I[illegible] sota Heart Association, and the Graduate School, University of Minnesota.
** With the technical assistance of H Louise Jernberg and H. Ardelle Mead

extremity was to decrease the foot-to-groin venous circulation time an average of 57 per cent over that obtained with the limb horizontal and 35 per cent over the average venous circulation time with the limb elevated.

COMMENT

The rate of venous flow in the lower extremities can be increased by (1) t ... elastic compression bandage to the horizontal limb; ... level, (3) the application ... extremity. The application of an elastic bandage ... ars to have a greater effect on the venous flow rate than does elevation ... extremity, and the application of an elastic bandage to the elevated limb appears to result in the greatest decrease in the venous circulation time.

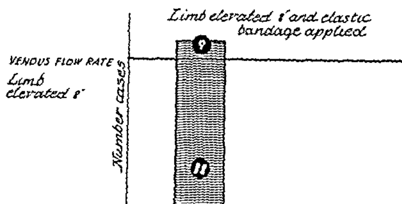


Fig 7. Venous circulation time in lower extremities Effect of application of elastic bandage to elevated limb

On the assumption that venous stasis in the lower extremities is a con- t ... factor to the development of venous thrombosis, these studies may ... the effect of muscular contraction on the ... in this study, it has ... in prevent- ing venous stasis ... ver, for patients confined to bed for significant period ... exercise of the lower extremities is not feasible, the venous flow rate in the lower limb may be improved by elevation of the extremities and application of elastic bandages

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Table 3 summarizes briefly the results observed on the four animals in chronic heart failure during the two periods of metabolism study before and after subtotal adrenalectomy. Unfortunately, animal No. 4 died before adrenalectomy could be performed. At autopsy severe peripheral edema, ascites and bilateral hydrothorax were found. Of special interest in comparing these two groups of figures is the appreciable increase in sodium and water diuresis, weight decline and increase in plasma proteins occurring

Table 2. Incidence of Ascites in Dogs with Chronic Heart Failure with Subtotal Adrenalectomies

NO. DOGS	MEAN RAP*	ASCITES	PER CENT
20	+7 85	3	15

* Range of RAP = +4 to +13.5 mm Hg.

in all animals after subtotal adrenalectomy. Dog No. 2, which showed the least change post-adrenalectomy, was in mild failure at the beginning of the experiment.

DISCUSSION

Undoubtedly multiple factors play a role in the fluid and salt retention of chronic cardiac failure. The effects of hydrostatic pressure and intra- and extravascular colloid osmotic pressure on the formation of ascitic and

Table 3. Metabolism Study of Dogs in Chronic Heart Failure before and after Subtotal Adrenalectomy

DOG NO.		WATER INTAKE CC /24 HR.	URINE OUTPUT CC./24 HR	URINARY SODIUM GM /24 HR	PLASMA PROTEINS GM %	RT. ATRIAL PRESSURE MM HG	MEAN WT POUNDS
1	Before	1607	132	0.2	3 1	+9	47.8
	After	1333	329	2 97	5 8	+10	38 2
2	Before	1825	355	1 5	5 70	+9	46 8
	After	1677	479	1.96	6 55	+9	44.9
3	Before	1261	198	0 7	3 5	+10	37 5
	After	1489	468	2 42	6 7	+11 5	26
4	Before	2655	1443	0 46	3 9	+15	50
	After	Died pre-adrenalectomy					

edema fluid are well known. There is also ample evidence that hormonal factors play a dominant role Tewell and Freeman[5] have shown in animal experiments that adrenalectomy and hypophysectomy causes reduced excretion of corticoids and that these were probably of adrenal origin. Hamilton[3] has stated that the ascites occurring in dogs with mitral stenosis can be reversed with adrenalectomy. Recently Davis et al.[2] have shown a diuresis of salt and water in adrenalectomized animals with ascites maintained on Doca. They also demonstrated that maintenance doses of Doca were the same in animals with ascites and in the same animals after diuresis. For ascites to develop increased doses of Doca over the maintenance dose were required. The quantity of sodium retained became progressively greater as the dose of Doca increased. They observed similar patterns in fecal excretion of electrolytes indicating that excessive amounts of cortical hormones

infundibular stenosis and a tricuspid valve insufficiency as previously described.[1, 4] Right atrial pressure measurements were made under local anesthesia by cardiac catheterization using strain gauge manometers and a Poly-viso recorder. Two series of observations were made. In the first series, the incidence of ascites in two groups of animals with elevated right atrial pressures with and without subtotal adrenalectomy were compared. Subtotal adrenalectomy consisted of removal of the right adrenal and one-half of the left adrenal, which was done in one stage about two weeks following the cardiac procedures referred to previously. Neither preoperative nor postoperative hormonal therapy was necessary to maintain the dogs. Following the operative procedures, the animals were catheterized on several occasions and also observed for the development of ascites. Ascites was detected by weight gain, abdominal palpation and paracentesis

In the second series of experiments, metabolism studies were carried out on a single group of four animals with chronic heart failure before and after subtotal adrenalectomy. Dogs with elevated right atrial pressures and ascites were first tapped dry of ascitic fluid, then placed in metabolism cages for continuous study over two-week periods Water was allowed ad libitum and the daily consumption recorded. Measured fixed quantities of food and salt were given daily. The ration consisted of one pound of dry food* plus five grams of salt in capsule form in divided doses. This amount of salt was well tolerated by the animals Weights, urine volumes and urinary sodium excretions were recorded daily. Plasma protein determinations were made three or four times during each two-week study period Measurements of urinary sodium were determined by flame photometry Upon completion of the control study, subtotal adrenalectomy was performed. After a recovery period of two to three weeks during which time the animals were given the routine kennel care and feeding, the metabolism studies as outlined above were repeated

RESULTS

The incidence of ascites in the two groups of animals with elevated right atrial pressures, one group with and the other without subtotal adrenalectomies, is shown in Tables 1 and 2 In the control group of twenty-one dogs without adrenalectomies (Table 1), fourteen, or 66 6 per cent, developed

Table 1 Incidence of Ascites in Dogs with Chronic Heart Failure

NO DOGS	MEAN RAP*	ASCITES	PER CENT
21	+7 28	14	66 6

* RAP = Right atrial pressure in mm Hg Range of RAP = +5 to +12 5 mm Hg

ascites. The mean right atrial pressure for the group was +7.28 mm Hg, with the range being +5 to +12 5 mm. Hg.

In the group of twenty dogs with subtotal adrenalectomies (Table 2), three, or 15 per cent, developed ascites The mean right atrial pressure for the group was +7 85 mm Hg, with the range being +4 to +13 5 mm. Hg. The two groups of animals are seen to be almost identical with regard to degree of elevation of right atrial pressures. As a rule ascites appeared more promptly and to a greater degree in the control group.

* Fromm Dog Meal, manufactured by Federal Foods, Inc, Thiensville, Wisconsin.

pressure measurements in human cases of coarctation demonstrated a diastolic hypertension in the vascular bed distal to the aortic stenosis. These and other reports have given rise to a second theory, namely, that the hypertension is renal in origin.

Many attempts have been made to resolve the problem by studies of peripheral resistance, and determinations of renal flow and function. The results of these studies have been conflicting. To support the mechanical theory, an increase in pressure during acute occlusion of the aorta has been reported, but these acute experiments have not duplicated the clinical condition.

We have attempted to answer the question by transplanting all renal tissue to a site proximal to the coarctation, and observing the effect on the hypertension. This approach to the problem is identical to that of Scott and his co-workers,[3, 4] who reported on their method prior to the completion of our studies. This is a confirmation of their findings, plus other pertinent

PLAN OF EXPERIMENT

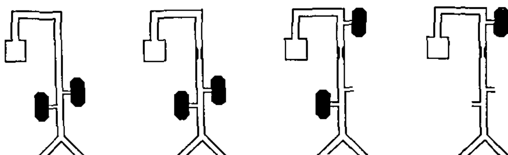


Fig 1.

observations, and is further evidence that the hypertension of coarctation is renal in origin.

The plan of the experiment is illustrated diagrammatically in Figure I.

METHODS

Leptospira infection in the casual dog population is high, with resultant renal disease, and possibly hypertension.[5] All our dogs were tested for this infection and those with a high titre, or hypertension, were excluded.

We found Sealy and McSwain's method[6] of producing coarctation of the aorta very satisfactory, and used it throughout the experiment. A Lucite tube with an hourglass constriction is tied into the divided thoracic aorta, with a resultant reduction in cross-sectional area of about 85 per cent. If moderate-sized dogs are selected hypertension invariably results in three to ten weeks If the dogs are small hypertension may not develop, as the cross-sectional area must be reduced by at least 75 per cent. If on the other hand the dogs are very large, the incidence of paraplegia will be high.

There are several technical details that must be observed if the incidence of secondary hemorrhage, or thrombosis in the tube, is to be kept low. The aorta is divided between clamps and the ends slipped over the Lucite tube, which has three outside grooves on either end. Damage to the intima by the

are necessary to produce low sodium and high potassium electrolyte pattern during ascites formation

In the experimental data presented here, subtotal adrenalectomy appears to protect the animal with chronic heart failure from ascites formation by preventing salt and water retention. It is most important to note that in these animals the remaining adrenal tissue is sufficient to maintain normal health, but is incapable of producing retention of sodium necessary in forming ascites.

SUMMARY AND CONCLUSIONS

1. Two groups of dogs with chronic heart failure with and without subtotal adrenalectomy are compared as to incidence of ascites.
2. The incidence of ascites in the control group was 66.6 per cent in contrast to an incidence of 15 per cent in the adrenalectomized group.
3. A metabolism study on a third group of animals with ascites observed before and after subtotal adrenalectomy showed increased sodium and water excretion following subtotal adrenalectomy.
4. Subtotal adrenalectomy appears to afford protection against ascites formation in animals with chronic heart failure. Hormonal replacement therapy has not been necessary to maintain good health in these animals.

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THE RENAL FACTOR IN THE HYPERTENSION OF EXPERIMENTAL COARCTATION OF THE AORTA*

R. C HARRISON AND J. D M. ALTON

Two conflicting theories have been advanced to explain the hypertension in association with coarctation of the aorta The oldest and most obvious explanation is that it is the result of mechanical obstruction In 1939, Goldblatt[1] demonstrated that renal artery compression in dogs resulted in hypertension, and two years later Steele[2] reported that accurate intra-arterial

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DISCUSSION

We are unable to explain why the hypertension took some weeks to become established, but fell within days when the loin kidney was removed. If the development of the hypertension was on a mechanical basis, it would have been maximal following coarctation, and would have then subsided as collaterals developed.

Although this experiment demonstrates that the kidney is involved in the production of hypertension, it does not necessarily follow that it is entirely

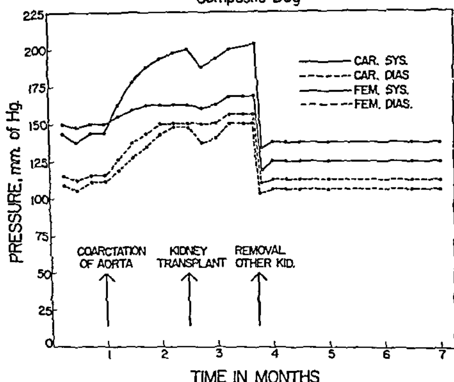


Fig 2.

responsible. It is fair to state that the kidney appears to initiate the hypertension Once established, it is reversible if the renal tissue is removed from the vascular bed distal to the constriction. We repeated this experiment in 2 dogs that had been hypertensive for over a year, and the results were identical. The mechanism was apparently still primarily renal.

Coarctation of the aorta appears to affect renal circulation much like the Goldblatt clamp, with a similar result. What change in hemodynamics occurs that apparently stimulates the kidney to produce pressor substances? It has been noted that following coarctation the average *increase in femoral* systolic pressure was 12 per cent, while the average increase in femoral diastolic pressure was 26 per cent. The kidney is *certainly* not in a hypotensive environment, and this cannot be the explanation *for its* pressor production. Its environment is actually hypertensive. Studies of renal blood flow in human cases of coarctation of the aorta are conflicting,[7-9] but if there

clamps will result in a thrombus which occludes the tube, with subsequent paraplegia We found Potts clamps to be superior. The divided aorta must be tied over the tube so that it does not slip off, with primary hemorrhage, yet the aorta must not be devitalized under the ligature, or secondary hemorrhage will occur. By using a tight ligature in the innermost grooves, and a large loose ligature in the outermost grooves, this problem was solved after several fatalities.

Pressures in the femoral and subcutaneously transposed carotid arteries were recorded through a 21 gauge needle by a Sanborn Electromanometer and Visocardiette. They were done under Pentothal anesthesia and repeated frequently between each stage of the experiment

When hypertension was well established and stable, the left kidney was transplanted to the left side of the neck. An end to end anastomosis was effected between the renal and common carotid arteries, and between the renal and external jugular veins, the ureter being brought through the neck skin as a cutaneous ureterostomy.

After some weeks, when pressures had again stabilized, the right kidney was removed, so that all functioning renal tissue was now proximal to the coarctation.

RESULTS

Forty-one dogs had thoracotomies for the production of coarctation, and of these 21 survived and became hypertensive Most of the deaths were due to primary or secondary hemorrhage, and occurred early in the series. A dog was not considered hypertensive unless the carotid systolic pressure exceeded 200 mm. Hg.

Eight of these hypertensive dogs were kept as controls, and were still alive and hypertensive after one year. During this period of time their hypertension has, in fact, increased slightly

Thirteen dogs had a kidney transplanted to the neck, with three deaths The 10 surviving dogs later had the second kidney removed from the loin In all, pressures remained hypertensive until the renal tissue distal to the coarctation was removed, when they returned to pre-coarctation levels The sequence of events is shown in Figure 2. Five of this group are still alive and well, with no return of the hypertension and no significant increase in blood urea nitrogen The longest survival has had a neck kidney for 16 months, and this has been his only kidney for 14 months

Although it took some weeks for pressures to become hypertensive following coarctation, they fell to pre-coarctation levels within a few days when the second kidney was removed.

All pressures did not change to the same degree, owing to the dampening effect of the aortic constriction on the femoral pulse curve. All pressures returned to their preoperative levels or lower with the coarctation still present.

The average change in pressure in the 10 dogs following coarctation only was carotid systolic +34 6 per cent, carotid diastolic +33 8 per cent, femoral systolic +12.2 per cent, and femoral diastolic +26 6 per cent.

The average change in pressure following coarctation plus kidney transplant plus contralateral nephrectomy was carotid systolic —2.3 per cent, carotid diastolic —3 4 per cent, femoral systolic —17 8 per cent, and femoral diastolic —4 8 per cent.

THE AUGMENTATION OF PERIPHERAL ARTERIAL BLOOD FLOW BY THE USE OF A VALVE*

ADRIAN KANTROWITZ AND ALAN LERRICK**

The modern era in the quantitative measurement of phasic blood flow patterns may be said to have begun with the development of the differential pressure recorders. Of these, the orifice meter has, perhaps, been used most extensively by investigators.[1,2] In 1943, Shipley, Gregg, and Schroeder reported their studies of blood flow patterns in peripheral arteries using this instrument.[2] They and other investigators have shown that in many of the larger vessels such as the common carotid, axillary, coronary, and femoral arteries there is a significant backflow which occurs in the latter part of systole.[2-5]

Figure 1 is a reconstruction of the femoral arterial pressure pulse, *P*, and the flow pattern, *F*, in the femoral artery of an anesthetized dog. The flow

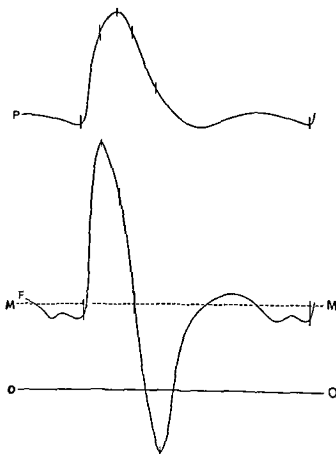


Fig 1 Simultaneous pressure and flow curves from a dog's femoral artery. *P*, Arterial pressure, *F*, arterial flow, dotted line *MM*, mean flow, line *OO*, zero flow. After Shipley, Gregg, and Schroeder.[2]

* From the S... Research Laboratory ... New York. This work was s...

** ... n C Ferreira and Mr. Alphonso Ivan Henry for their technical assistance

is a reduction in arterial flow it is not of great magnitude. Certainly there is no renal ischemia as such; the change in hemodynamics is much more subtle

It has been postulated by Page,[10] and some evidence has been obtained, that the critical change in hemodynamics is a reduction in renal artery pulse pressure. In our series of 10 dogs the average femoral pulse pressure was reduced by the coarctation from 36 mm. Hg preoperatively to 16 mm. Hg postoperatively. In one dog, however, it was not reduced at all, and only to a moderate degree in a second The development of hypertension in these two animals was as marked as in the remainder of the series. Our experiment does not lend support to this theory.

We are unable to define the difference in the hemodynamics in the two zones cephalad and caudad to the coarctation.

SUMMARY

1 Experimental coarctation of the aorta with hypertension has been produced in a series of dogs which resembles clinical coarctation.

2. Although the precise mechanism leading to the production of the hypertension is still to be determined, it is initiated by a renal mechanism

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have done. By clos-
to pass through the
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pressures and flows through the dog's femoral artery, with and without a valve in the circuit. The experimental and control observations were made

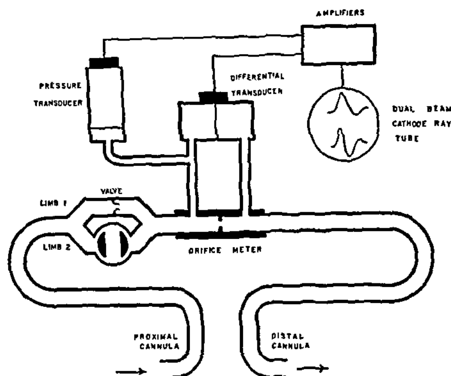


Fig. 2 Diagram of experimental setup for femoral arterial inflow studies (see text).

a few seconds apart. Thus, it was possible to compare the flows through the dog's leg with a valve in the femoral artery, to the flow without a valve, in the same animal under almost identical conditions.

RESULTS

It was possible to make 47 separate, controlled observations in these animals. In all cases, the mean flow was greater into the dog's leg with the valve in place, as compared to the control observations when the valve was not in place Sections of a typical record of one of these experiments, using this setup, are shown here in Figure 3. The upper curves depict the femoral artery pressures. The middle curves are the recorded flows in cc. per minute, and the lower curves are reconstructions of the recorded flow on linear coordinates. Section A shows the simultaneous pressure and flow curves under the control conditions. Section B shows the simultaneous pressure and flow curves with the valve in place. Section B was recorded 7 seconds after section A. The
section B is
A was 25 cc ... and the mean flow in section B was 38 cc. per minute, an increase of 52 per cent. One can see in section B, where the valve is in place, th
the pressure

pattern represents the direction and velocity with which blood passes a point in the femoral artery. The dotted line, *MM*, represents the mean flow and line *OO* represents 0 flow. The pressure and flow start to rise simultaneously. The flow reaches its maximum forward velocity before the pressure reaches its peak Then the forward velocity of the flow begins to decrease. After the peak in pressure has been reached and begins to fall, the forward velocity of the blood flow continues to fall rapidly until it reaches zero. It then reverses its direction, and during the latter part of systole and the beginning of diastole, the blood flows toward the heart in this artery. In the early part of diastole, this backward flow is reversed and the blood again resumes its forward direction, i.e, away from the heart. What this means essentially is that during the early part of systole, when the pressure is rising very rapidly in the proximal portions of the femoral artery, more blood is forced into the arterial system of the leg than can run off peripherally through the capillary bed. Therefore, during the latter part of systole when the pressure is dropping centrally some blood must reflux back up into the abdominal aorta

It occurred to one of us (A K.) that if it would be possible to eliminate or, at least, reduce this backflow without affecting the forward flow appreciably, the mean forward flow would be increased. If the blood were to be trapped by some sort of a check valve in the distended femoral arterial system, after it had flowed in under this high pressure, the total mean forward flow should be increased by the amount that was trapped and would normally reflux centrally. If the valve were to close during the latter part of systole, when the pressure is falling rapidly (owing to the reflux of blood centrally), one would expect that the diastolic pressure would be maintained. This would be similar to what happens at the aortic valve when the diastolic pressure in the aorta is maintained at higher levels than that in the left ventricle. If the diastolic pressure were maintained, then the mean pressure would be increased From Poiseuille's law[6] one would expect that if the mean pressure in the leg were maintained at a higher level, then the mean flow should be greater.

METHOD

A series of acute experiments was done in anesthetized adult mongrel dogs in order to test the validity of this hypothesis. The experimental setup is diagrammatically illustrated in Figure 2 The left femoral artery was dissected free for a length of about 5 cm. in the groin. The artery was then severed and 2 cannulae were inserted. one in the proximal end of the artery in such a position as to receive all of the blood from the femoral artery, the other in the distal segment of the artery in such a position as to deliver all of the blood to the peripheral portion of the animal's leg. From the proximal cannula the blood was led to a Y-tube In one limb of the Y-tube, a fast-acting valve was inserted in such a fashion so as to permit blood to flow only in the direction toward the periphery, that is, it did not permit any backflow. The other limb of the Y-tube contained a stopcock arranged so it was possible to shut it off at will. A second Y-tube rejoined the streams and the blood was led to an orifice meter. At this point the pressures and the flows were recorded. From the orifice meter, the blood was returned to the distal cannula and continued on its way peripherally to the tissues of the leg. By leaving the stopcock open in limb 2, it was possible to study the normal

normal flows could be recorded by opening the stopcock, and flows with a valve in place could be recorded by closing that stopcock. Eighteen separate observations were made. Again in all cases, the flow was greater with the valve in place than under the control conditions.

The record of a typical experiment is illustrated in Figure 5. The upper tracings are the pressures in millimeters of mercury. The sloping line across the lower portion of section *B* is the flow curve. Section *A* is the contour of the pressure curve with the valve in place. The point can be observed at which the valve closes with the consequent maintenance of the diastolic pressures at higher levels than the control pressure curve seen in section *C*. Section *B* was recorded at a slower paper speed than that in sections *A* and *C*. In the first half of section *B*, the valve is in place. The valve is removed

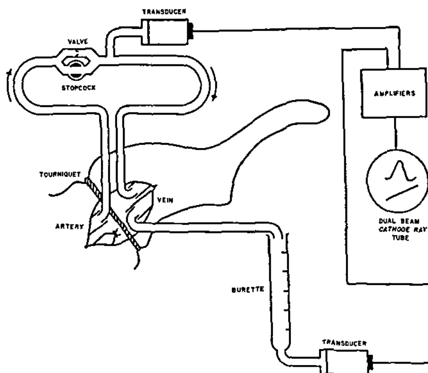


Fig 4 Diagram of experimental setup for femoral vein outflow studies (see text)

(by opening the stopcock) at the point designated by the vertical broken line. It can be seen that the slope of the flow curve changes sharply at this point. In the first part the slope of the flow curve rises rapidly, indicating a greater outflow from the dog's leg with the valve in place. In the second portion of section *B* the slope does not rise as rapidly, indicating a lesser outflow from the dog's leg when the valve is removed. The mean pressure in section *A* is 146 mm. Hg; the mean pressure in section *C* is 126 mm. Hg. The flow in the first portion of section *B* is 53 cc./minute and the flow in the second half of section *B* is 36 cc./minute, an increase of 47 per cent.

DISCUSSION

It may be feasible to apply these findings in the treatment of human peripheral vascular disease where it is felt that the pathologic process, for the most part, involves the main stem arteries and that the smaller branches

a higher level throughout the rest of diastole than in the control curve. In the flow curve, this has the consequence of decreasing considerably the ean forward flow. rder to be certain inserting a valve into the artery, but that the venous outflow is also increased. To do this, the setup diagrammatically illustrated in Figure 4 was used. It is similar to the arrangement previously used except that the orifice meter and differential transducer were removed and the flow was studied by collecting the blood *returning from the leg via the femoral vein* A *steel cable tourniquet* was applied around the animal's leg in these experiments in such a fashion as to

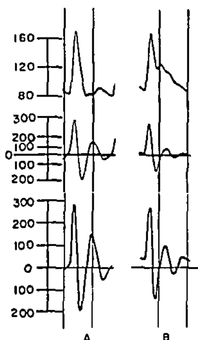


Fig 3 Segments from record in dog No. 6-20. Upper curves represent femoral artery pressures in mm Hg, middle curves, phasic flow as recorded with an orifice meter in cc per minute, lower curves, reconstructions of phasic flow curves on linear ordinates Section *A* is control flow, section *B* is flow with valve in femoral artery circuit Time lines represent 0 02 seconds Flow is 52 per cent greater with valve in circuit

assure that all blood entered the leg through the femoral artery and external blood circuit and, after passing through the leg, was able to leave only through the femoral vein which was cannulated. All blood collected by the cannula in the femoral vein was allowed to pour freely into a 25 cc burette. As the blood level in the burette rose, the pressure on the transducer at the bottom of the burette was increased and this was recorded on the cathode ray tube. The rate of increase of the weight of the blood in the burette was proportional to the rate of flow. At no time was more than 25 cc. of blood removed from the animal. After an observation, all blood collected in the burette was returned to the animal by way of an intravenous infusion in the other leg. *It was possible to record pressures, as was done in the previous* experiment, by placing a transducer in the external arterial circuit. Again

known that a considerable backflow normally exists in some arteries of the experimental animal.

2. It is suggested that by eliminating the backflow in the femoral artery by means of a check valve, the mean forward flow should be increased.

3. This hypothesis was tested in a series of 23 acute experiments in anesthetized dogs.

4. In 8 dogs, the arterial inflow was studied with and without a valve in site. In 47 separate controlled observations, it was found that the arterial inflow was increased anywhere from 6 to 52 per cent.

5. In 3 other animals, the venous outflow from the dog's leg was studied in 18 separate controlled observations with and without a valve in the femoral artery. Increases of the venous outflow were observed in all cases.

6. Finally, some reasons are presented why this may be of value in the treatment of human peripheral arterial disease.

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POLARIGRAPHIC STUDIES ON CIRCULATION IN THE DOG*

CHARLES C. WOLFERTH, JR., ANDREW BOYD, JR., AND WILLIAM T. FITTS, JR.

In these investigations an attempt is being made (1) to find a *simple in vivo* method of observing the oxygen tension in isolated skeletal muscle and liver, (2) to correlate measured oxygen tension with measured blood flow in skeletal muscle, and (3) to present a method of observing *some* of the changes in the oxygen tension in liver, skeletal muscle and skin in dogs with normal and altered hepatic circulations following acute blood loss.

RELATIONSHIP OF OXYGEN TENSION TO BLOOD FLOW IN ISOLATED DENERVATED RESTING SKELETAL MUSCLE

The Davies and Brink electrode[1-4] was modified for use in muscle and

* From the Harrison Department of Surgical Research, School of Medicine, University of Pennsylvania, Philadelphia.

which are responsible for carrying the collateral supply of blood are often free of disease.[7] If it were possible to place the valve in such a way as to increase the blood flow through the collateral system, then this method might be of some usefulness. Certainly, if one were to place the valve in the common iliac artery above its bifurcation into the hypogastric and the common femoral, one would then be in a position to affect the pressures in most of the collateral vessels in the leg, since they arise from branches below this point. Plethysmographic studies[8] on individuals suffering from arteriosclerotic peripheral vascular disease suggest that some elasticity still exists in their arterial tree. Indeed, measurable backflow can often be demon-

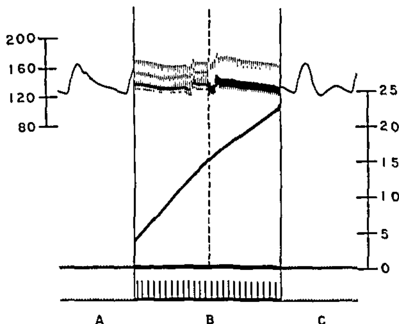


Fig 5 Segments from record in dog No 6-23 Upper curves represent femoral artery pressure in mm Hg, sloping line represents venous outflow in cc Section *A*, contour of pressure curve with valve in femoral artery circuit, first half of section *B*, valve in femoral artery circuit, second half of section *B*, control flow, valve removed from circuit at point indicated by vertical broken line, section *C*, contour of pressure curve without valve in femoral artery circuit Time marks in section *B*, 1 second. Flow 47 per cent greater with valve in circuit.

strated. Therefore, one would expect that the human with peripheral vascular disease should not react any differently than the experimental animal when a valve is placed in his femoral artery.

Finally, the observation should be made that if it is possible to improve

animals. A valve which may have usefulness in human (adult and pediatric) vascular problems is being developed.

SUMMARY

1. Since the development of modern phasic inflow meters, it has been

Table 1.

TIME (MIN.)	BLOOD FLOW (CC/MIN.)	MUSCLE OXYGEN TENSION OF INDIVIDUAL ELECTRODES (EXPRESSED IN GALVANOMETER UNITS)									AVERAGE
0	49	44	42	64	63	41	44	55	31	51	*48*
20	20	33	45	65	60	35	35	45	31	41	*43*
40	41	36	46	70	61	41	37	47	33	43	*45*
55	19	34	45	62	57	33	36	40	32	42	*42*
60	18	31	42	61	48	34	33	37	30	38	*39*
70	18	34	44	60	56	33	30	37	29	35	*40*
80	17	31	40	62	56	32	32	36	29	35	*39*
90	15	30	45	58	50	32	32	35	28	35	*38*
100	14	29	44	58	48	30	28	32	27	33	*37*
120	13	28	42	56	48	29	26	31	28	31	*35*
130	14	29	40	57	49	28	29	31	24	35	*36*
140	14	29	40	57	50	30	31	32	26	30	*36*
150	12	27	39	54	47	27	29	28	24	25	*33*
160	9	26	36	51	40	25	27	25	22	21	*30*
180	8	24	35	49	37	23	26	22	20	18	*28*
210	3	20	32	46	33	29	22	19	16	16	*25*

Table 2.

TIME (MIN)	BLOOD FLOW (CC/MIN)	MUSCLE OXYGEN TENSION OF INDIVIDUAL ELECTRODES (EXPRESSED IN GALVANOMETER UNITS)						
0	35	44	46	35	51	42	54	40
20	25	36	39	28	42	32	47	35
40	33	42	43	31	48	38	53	39

2. In the early phase of an experment, if 100 per cent oxygen was inhaled, the muscle oxygen tension rose sharply. In the later phase of the experiment, however, when the anımal was in shock, lıttle or no rise of the muscle oxygen tensıon occurred ın spite of the same blood flow. Yet, under these circumstances, the venous blood, whıch had appeared quite dark, would become bnght red when oxygen was admınistered.

Table 3.

TIME (MIN)	BLOOD FLOW (CC/MIN)	MUSCLE OXYGEN TENSION OF INDIVIDUAL ELECTRODES (EXPRESSED IN GALVANOMETER UNITS)								AVERAGE
10	30	24	117	65	61	48	55	59	55	*60*
20	29	24	98	62	56	48	57	50	60	*57*
30	100% Oxygen ınhalatıon									
40	25	53	162	183	123	132	136	168	96	*132*
50	24	40	159	207	128	108	147	141	108	*130*
60	100% Oxygen off									
120	26	12	46	40	35	22	37	36	43	33
125	100% Oxygen ınhalatıon									
130	20	12	46	50	30	17	32	39	78	38

3. When the femoral artery was occluded ın the isolated leg, the oxygen tensıon readings in the muscle rapıdly fell to almost zero levels. When the artery was reopened, the readings rose again.

4. Venous pressures of 10, 20 and 30 cm of blood were produced by appropriate elevatıon of the femoral vein catheter during some of the

liver as follows: An alloy of 87 per cent platinum 13 per cent ruthenium wire was given multiple thin coatings of a thermosetting plastic.* A 1 to 2 mm uninsulated cone-shaped tip of wire was then exposed by grinding off the plastic. The electrode was soldered to a polyethylene-sheathed copper wire which served as the lead. The recording device is similar to that described by Davies and Brink.

Experimental Procedure

Healthy adult mongrel dogs were anesthetized with intravenous sodium pentobarbital. The skin was incised and removed completely from the upper thigh down to a point where a heavy ligature was tied around the leg to prevent distal blood flow beyond what will be the isolated muscle area. The leg muscles down to the femur were divided and ligated just below the groin The periosteum of the femur was incised at the same level completely around the bone. The sciatic and femoral nerves were divided. Heparin sodium was administered hourly through the intravenous catheter in the femoral vein of the opposite leg. Electrodes were placed at random depths in the isolated muscles. The femoral artery on the side of the isolated muscle was exposed and the adventitia stripped from it over a distance of 2 to 3 cm , thus interrupting most of the remaining sympathetic fibers. The femoral vein on the same side was opened and a polyvinyl catheter was inserted caudad into and draining the venous return of the isolated muscle; the proximal end of the vein was ligated The opposite end of the catheter

volume was kept relatively constant

Galvanometer readings were made 20 seconds after polarization of the electrode. 100 per cent oxygen could be administered through an endotracheal catheter at 6 liters/min At all other times ambient air was delivered at 6 liters/min through the same catheter on a demand basis.

Results

Ten experiments were performed. Although there was some quantitative difference between individual experiments, the over-all pattern of the electrode responses was quite similar The results are best illustrated by typical protocols.

1. A definite pattern of electrode responses to amount of blood flow was demonstrated The greatest blood flow per unit time in the isolated muscle occurred just after the venous catheter was inserted and at the initial determination of blood flow (possibly because of venous obstruction while the catheter was being inserted), subsequently the flow decreased. By manipulation of the amount of circulating blood volume, however, the flow was brought back to nearly the initial level It is to be emphasized that in the early part of an experiment, when the blood flow was returned almost to initial levels, the oxygen tension reading would closely approximate the initial readings obtained when the animal was breathing ambient air.

* "Araldite."

blood pressure at 70 mm. Hg. Endotracheal catheters were inserted so that the effects of breathing 100 per cent oxygen could be determined.

Results

Seventeen experiments were performed. Ten dogs with normal circulations and seven dogs, following recovery of at least one month from ligation [illegible] arteries, were studied. [illegible] tension readings at normo- [illegible]ing ambient air at 6 liters/ min. through a semi-closed breathing apparatus. 100 per cent oxygen was then administered at 6 liters/min. through the same semi-closed breathing apparatus.

The electrode readings given in the tables represent average values for each tissue (10 in the liver, 5 in skeletal muscle and 5 in skin). It was noted

Table 5. Intact Hepatic Circulation

TIME (MIN)	BLOOD PRESSURE	AVERAGE OXYGEN TENSION OF: LIVER (EXPRESSED IN GALVANOMETER UNITS)	MUSCLE	SKIN
0	130	50	57	74
20	130	50	56	73
	100% Oxygen inhalation			
35	127	125	79	107
	Oxygen off			
45	125	57	53	77
65	70	55	35	51
95	70	55	32	49
125	70	53	33	50
	100% Oxygen inhalation			
140	70	82	31	48
	Oxygen off			
175	122	53	50	72
185	125	55	51	71
	100% Oxygen inhalation			
200	126	124	80	94

that with the administration of 100 per cent oxygen at normotensive pressure levels, there was a 150 per cent rise in the oxygen tension of the liver, while the oxygen tension of the skin and skeletal muscle rose about 50 per cent. The animal was then bled rapidly to a mean arterial pressure of 70 mm. Hg and maintained at this pressure for one hour or more. During such periods oxygen tensions of the skin, skeletal muscle and liver were recorded With the animal breathing ambient air at 6 liters/min the average recordings showed a 30 to 50 per cent drop from the normotensive values in the oxygen tension of skin and skeletal muscle, while the liver oxygen tension was the same as values obtained at normotensive levels on ambient air. It was observed that when the hypotensive animal breathed 100 per cent oxygen at 6 liters/min. there was a rise of 40 to 50 per cent in the oxygen tension of the liver from the values obtained at the same pressure levels when ambient air at 6 liters/min. was breathed However, in the same experiments the oxygen tension of the skin and skeletal muscle either showed no change

experiments. There did not seem to be any significant change in the blood flow or oxygen tension of the muscles at these various venous pressures.

Summary

1. A practically linear relationship of oxygen tension to blood flow as measured polarigraphically over a wide range in isolated, denervated resting skeletal muscle of the dog has been shown. This, we believe, demonstrates the validity of the polarigraphic method as a testing procedure for variation in blood flow in resting muscle under controlled conditions.

2. Administration of 100 per cent oxygen through an endotracheal catheter produces a sharp rise in the oxygen tension of skeletal muscle if the dog is normotensive but not if it is in shock from hemorrhage, even though muscle blood flow is approximately the same.

3 The finding that increased venous pressure does not alter the oxygen tension or the blood flow through the isolated muscle supports the idea that higher venous pressures do not, within the ranges studied, in themselves enlarge capillary beds and cause capillary pooling.

Table 4.

TIME (MIN)	BLOOD FLOW (CC/MIN)	MUSCLE OXYGEN TENSION OF INDIVIDUAL ELECTRODES (EXPRESSED IN GALVANOMETER UNITS)								AVERAGE
150	18	14	25	20	18	11	15	11	8	*15*
155	Artery occluded									
160	0	2	4	3	5	4	4	5	2	*4*
165	Occlusion removed									
170	22	15	30	23	17	15	14	13	11	*17*

OXYGEN TENSION STUDIES IN THE LIVER, SKELETAL MUSCLE AND SKIN OF DOGS. EFFECTS OF HYPOTENSION PRODUCED BY HEMORRHAGE

Oxygen tension readings were made in the liver, skeletal muscle and skin in a series of dogs before and after they were subjected to hemorrhagic shock. A comparison was made between liver with normal vascular channels and following chronic ligation of the hepatic artery.

Experimental Procedure

Healthy adult mongrel dogs were anesthetized as described above. Liver was exposed through a midline subcostal incision. The animal was given heparin intravenously. A polyvinyl catheter was placed in the abdominal aorta via the femoral artery. This catheter was in turn connected to a reservoir system which was vertically movable. By suitable adjustment of the height of the reservoir above the dog, blood pressure was maintained at a desired level of hypotension by allowing the animal to bleed into the reservoir. The mechanism is self regulatory at a desired pressure and will maintain blood pressure quite constant, either by bleeding additional amounts into the reservoir or by blood returning to the circulation from the reservoir. Electrodes were placed in skin, skeletal muscle and liver. Oxygen tension readings were made in the manner described above, first at normotensive levels and then following hemorrhage and maintenance of mean arterial

to hemorrhage. This is shown by the early and rapid fall in the muscle oxygen tension, even before a decrease in blood pressure is observed.

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MEASUREMENT OF AMBULATORY VENOUS PRESSURE IN THE LOWER EXTREMITY*

KARL A. LOFGREN

Measurement of venous pressure in the lower extremity during ambulation is an accurate, objective test of venous function performed under normal physiologic conditions. The first direct measurement of venous pressure at the ankle during walking was reported in 1936 by Smirk,[1] who used a normal subject for his study. Since that time numerous investigators have studied the venous pressure in normal as well as abnormal venous conditions of the lower extremity. This test has been used at the Mayo Clinic during the past 3 years as an adjunct to our other clinical tests for venous function in certain selected cases.

INDICATIONS

The measurement of ambulatory venous pressure is not practical or necessary for most patients with venous insufficiency, as simpler clinical tests fill the requirements for a diagnosis. For incompetent superficial veins the compression test of Schwartz-Heyerdale, as modified by Myers, and the tourniquet test of Brodie-Trendelenburg are usually sufficient. The former test helps to determine the course of the incompetent vein by transmitting impacts up and down on compression of the vein below and then above. The latter test demonstrates incompetent superficial veins by the rapid backfilling of the empty vein on release of the tourniquet placed above.

A satisfactory clinical test is lacking for deep chronic venous insufficiency of the lower extremity. The diagnosis is based clinically on the presence of stasis changes such as edema, pigmentation, induration or ulceration of the skin and subcutaneous tissues in the dependent portion of the leg. For this group the measurement of ambulatory venous pressure is useful for it gives an objective appraisal with a numerical index of the venous function in the lower extremity. In borderline conditions when the history or findings are not clear or are equivocal, and in certain other conditions, such as lymphedema or static edema, which may simulate venous insufficiency clinically, the ambulatory venous pressure has been measured to settle the question whether actual venous insufficiency was present or not. Thus in certain selected cases an objective, accurate test for venous function is urgently

* From the Section of Peripheral Vein Surgery, Mayo Clinic and Mayo Foundation, Rochester, Minnesota.

or a slight decrease in contrast to the increase found in the liver. When the blood was restored to the circulation and the blood pressure had returned to normotensive levels, the initial pattern of oxygen tension changes just described could be repeated.

It was observed that during the active bleeding stage of the experiment, an appreciable drop in skeletal muscle oxygen tension, as high as 20 per cent, occurred even before a change in blood pressure was detected. No observations on skin and liver oxygen tension were made during such a period.

Chronic Ligation of the Hepatic Artery. Seven animals were studied at least one month after the ligation and division of both the hepatic and gastroduodenal arteries. A segment at least 1 cm. in length was removed from each artery. (Seven survived out of a total of eight prepared.) Postoperatively, the animals were given 300,000 units of procaine penicillin and 1 gm. of streptomycin parenterally daily for at least five days. The results followed the same pattern as described above in animals with intact hepatic circulations.

Table 6. Chronic Ligation of Hepatic and Gastroduodenal Arteries

		AVERAGE OXYGEN TENSION OF:		
TIME	BLOOD PRESSURE	LIVER (EXPRESSED IN GALVANOMETER UNITS)	MUSCLE	SKIN
0	135	47	55	70
20	135	48	55	71
	100% Oxygen inhalation			
35	130	120	80	105
	Oxygen off			
45	135	52	54	73
65	70	51	33	54
95	70	53	32	54
125	70	50	34	53
	100% Oxygen inhalation			
140	70	80	32	53
	Oxygen off			
175	125	55	50	64
185	125	56	51	63
	100% Oxygen inhalation			
200	125	118	78	100

Summary

Although this work is a preliminary report, certain findings seem apparent.

1. All dogs subjected to hypotension from acute blood loss showed a drop of skin and skeletal muscle oxygen tension.
2. All the dogs studied showed no drop in liver oxygen tension following acute blood loss.
3. Administration of oxygen during shock following acute hemorrhage would seem to protect the dog's liver, at least so far as its oxygen tension is concerned.
4. If the animal survives ligation of the hepatic artery, the portal vein or some arterial regeneration from sources other than the hepatic artery is capable of maintaining liver oxygen tension.
5. The muscle vascular bed appears to be one of the earliest to respond

to hemorrhage. This is shown by the early and rapid fall in the muscle oxygen tension, even before a decrease in blood pressure is observed.

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needed, and the measurement of ambulatory venous pressure fulfills this need.

APPARATUS AND TECHNIQUE

Two different measurements are made with the patient in the upright position: The first is made while the patient is standing at rest and the second while he is walking or rising up on the toes at a set rate. The pressure obtained during exercise is the all-important one for it has a direct relationship to the state of venous function in the lower extremity. A 21-gauge needle connected by polythene tubing to a strain-gauge manometer is inserted into a dorsal vein of the foot, usually into the terminal portion of the great saphenous vein The manometer and tubing are filled with physiologic saline solution to which is added heparin (2 cc. to 1000 cc. of saline solution). The manometer is essentially a chamber with a sensitive diaphragm connected to a Wheatstone bridge, which in turn registers any pressure changes on a galvanometer. Then the readings on the galvanometer are calibrated on an upright meter stick into centimeters of physiologic saline solution.

The resting venous pressure is approximately equal to a force that will support a vertical volumn of venous blood from the third anterior thoracic interspace to the point of venipuncture. This represents a hydrostatic pressure which is not [illegible]
ment of their fun [illegible]
patient's height. [illegible]
ages between 130 and 140 cm. of physiologic saline solution.

NORMAL FINDINGS

The ambulatory venous pressure is normally lowered to half or less of the resting pressure. In a group of 21 normal lower extremities studied, the average ambulatory venous pressure in the lower extremities was 80 cm. lower than the average resting venous pressure of 133 cm In 1 normal subject the resting venous pressure was 140 cm. and the ambulatory venous pressure was 70 cm. Sitting down and elevating the leg to the horizontal position lowered the resting pressure in direct proportion to the reduced vertical distance between the heart level and the point of venipuncture (Fig. 1).

PHYSIOLOGIC ASPECTS

The ambulatory venous pressure is directly dependent on the combined action of the venous valves and the calf muscles. The former prevents the column of venous blood from receding downward, and the latter aids the venous return by compressing and pumping the veins during muscular contraction If either or both of these two agents are impaired, the ambulatory pressure fails to reach its normally lower level and a state of elevated ambulatory pressure ensues. The most common example of this is seen in the postphlebitic limb when the valves in the deep veins have been damaged and as a consequence chronic venous stasis is present in the dependent portion of the leg. An example of impaired action by the calf muscles is seen in ankylosis of the knee or ankle when these muscles are partially or wholly immobilized and as a consequence edema and venous congestion often develop around and just above the ankle.

When chronic venous insufficiency is present, the venous pressure fails to

drop down to its normal lower level during exercise. The ambulatory pressure is abnormally high. This undoubtedly produces an imbalance between the hydrostatic and osmotic forces in the small venules and capillaries so that increased transudation of intravascular fluid into the interstitial spaces is apt to occur in the dependent portion of the lower extremity where the venous pressures also are the highest.

RESULTS IN CASES OF CHRONIC VENOUS INSUFFICIENCY

Of Deep Veins. A group of 11 patients with chronic insufficiency of the deep veins that followed deep thrombophlebitis were studied. The ambulatory venous pressure in these cases averaged only 7 cm. lower than the rest-

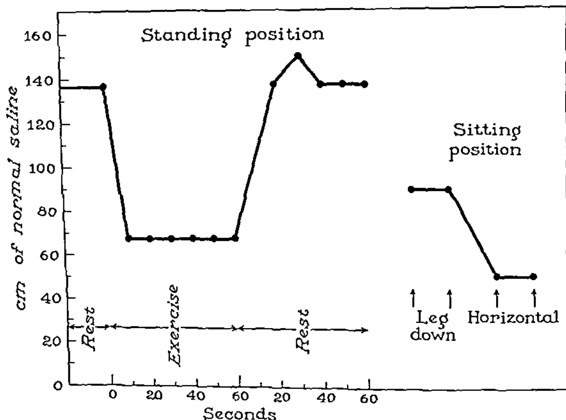


Fig 1. *Venous pressures of one normal subject, measured at the dorsum of the foot.*

pressure was 59 cm. less than the resting pressure, and he demonstrated extensive collateral veins over the lower part of the abdomen. All other patients had ambulatory pressures just slightly less than the resting venous pressures. Thus they exhibited marked elevation of the ambulatory pressures.

One patient, a man, 43 years of age, gave a history of having had iliofemoral phlebitis of the left leg 12 years previously which was complicated by a stasis ulcer. Treatment in the past had included skin grafting, and high ligation of the left great saphenous vein. At the time of admission to the

clinic he had a stasis ulcer above the left ankle. The resting venous pressure was 145 cm of saline solution and the ambulatory pressure was only 19 cm less. After skin graft and removal of a small superficial vein leading into the ulcer area, the ambulatory venous pressure remained unchanged.

With Cavernous Hemangioma. In another group of 6 patients who had extensive cavernous hemangiomas involving the lower extremity from hip to foot, the ambulatory venous pressure was studied. It averaged only 15 cm. lower than the resting pressure. This observation indicates a rather marked degree of venous insufficiency (Fig 3).

One patient, a woman, 38 years of age, had an extensive cavernous heman-

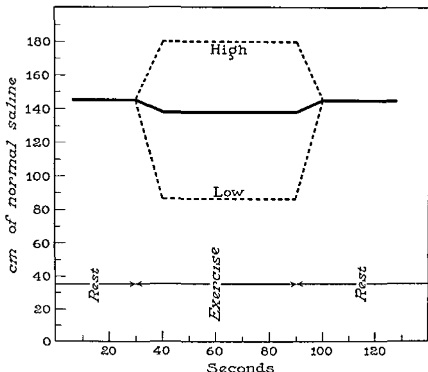


Fig 2 Average venous pressures of the lower extremities affected by chronic insufficiency of the deep veins 11 patients

gioma which involved the lateral portion of the left buttock, the thigh, and leg and foot The resting venous pressure was 130 cm and the venous pres- ... ambulatory ... icy. ... resent, the ... as high as ... ncompetent great saphenous veins studied, the ambulatory venous pressure averaged 46 cm. lower than the resting venous pressure, which was 134 cm. of physiologic saline solution when measured at the dorsum of the foot prior to operations on the veins. After extensive removal of the incompetent superficial veins by stripping from the dorsum of the foot to the saphenofemoral juncture and direct dissection of larger tributaries and perforating veins,

the ambulatory venous pressure averaged 84 cm. lower than the resting venous pressure. This was an improvement of 38 cm. and a restoration to normal levels (Fig. 4).

One patient, a woman 53 years of age, with a large incompetent great saphenous vein on the right had a resting venous pressure of 130 cm. The ambulatory venous pressure was only 25 cm. lower than the resting pressure

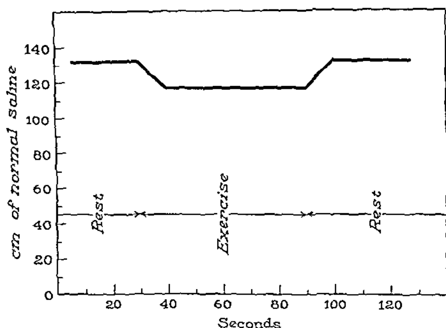


Fig 3 Average venous pressures of the lower extremities affected by cavernous hemangiomas. 6 patients

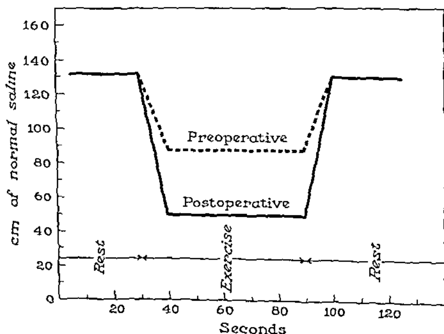


Fig. 4 Average venous pressures before and after complete stripping operations in 47 extremities (35 patients) with incompetent great saphenous veins.

in the involved extremity whereas it was 66 cm. lower in the normal left lower extremity. After a complete removal of the incompetent superficial vein by stripping and dissection, the ambulatory venous pressure in the extremity operated on was 70 cm. less than the resting pressure; thus it was down to a normal level.

In this group of 35 patients with incompetent superficial veins there were 8 with 13 recurrent incompetent great saphenous veins. The incompetency in these 13 extremities had recurred after previous local ligations at the groin. Prior to the extensive second operation, the ambulatory venous pressure in these extremities averaged 53 cm. less than the resting pressure. This was slightly better than the average for the entire group and indicated a slightly better venous function. After operation the ambulatory venous pressure was 86 cm. lower than the resting pressure, an improvement of 33 cm. as compared to the preoperative level and a return to normal levels (from a resting pressure of 134 to an ambulatory pressure of 48).

Four of the 35 patients with incompetent superficial veins had 4 incompetent small saphenous veins. The ambulatory venous pressure in the 4 affected extremities averaged 57 cm less than the resting venous pressure before operation Following stripping, the ambulatory venous pressure averaged 80 cm. less than the resting pressure, an improvement of 23 cm and a return to normal levels. The resting venous pressure was then 139 cm and the ambulatory pressure was 59 cm. In all instances in which uncomplicated incompetent superficial veins were removed surgically with complete stripping and dissection, the ambulatory venous pressure returned to normal levels. This finding was evidence of restoration to normal venous function in the lower extremity.

SUMMARY

The measurement of ambulatory venous pressure in the lower extremity is an accurate, objective test of venous function performed under normal physiologic conditions.

This test is very helpful in determining whether deep chronic venous insufficiency is present and the degree of insufficiency. It is especially helpful because of the lack of a reliable clinical test for this condition

This test has been used to demonstrate restoration of normal venous function in the lower extremity following extensive surgical treatment for varicose veins.

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A COMPARISON OF THORACOLUMBAR SYMPATHECTOMY AND BILATERAL ADRENALECTOMY-SYMPATHECTOMY IN THE TREATMENT OF ESSENTIAL HYPERTENSION*

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Because the end results of medical or conservative management of essential hypertension have been poorly documented in the past, it has been difficult to evaluate the effectiveness of surgical treatment of patients with this disease. Smithwick[1] has published data on a series of patients to indicate that there is an apparent extension of life expectancy of patients treated surgically by bilateral thoracolumbar sympathectomy.

During the past several years, the authors have published data[2,3] concerning the results of treatment of patients with essential hypertension by bilateral adrenalectomy-sympathectomy at the Hospital of the University of Pennsylvania It has been our impression that bilateral adrenalectomy-sympathectomy has been a more effective method of treatment of patients with this disease than bilateral thoracolumbar sympathectomy. Until recently, we have been unable to evaluate the results of these two procedures owing to an insufficient follow-up study. This report concerns two series of patients with essential hypertension treated surgically at the Hospital of the University of Pennsylvania. Seventy-six patients received bilateral thoracolumbar sympathectomy and have been followed from 3 to 7 years.[4] One hundred and twenty-five patients were treated by bilateral adrenalectomy-sympathectomy and have been followed from 0 to 4 years.[5]

In both series of patients, medical management of their hypertension had proven ineffective, and surgery was undertaken because of progression of vascular damage and symptomatology. These patients were evaluated pre- and postoperatively by members of the Hypertension Section, and careful estimations of the cardiac status, renal function and ocular fundi were made.

OPERATION

In the patients subjected to bilateral thoracolumbar sympathectomy, the three splanchic nerves were excised and the sympathetic chain was removed from the highest origin of the greater splanchnic nerve, which was usually located at the fifth or sixth thoracic ganglion, through the third lumbar ganglion.

The adrenalectomy-sympathectomy procedure included a resection of the sympathetic chain from T12 to, and including, L2. The greater and

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lesser splanchnic nerves were excised for a distance of about one inch below the diaphragm. Initially a subtotal resection of adrenal tissue was done, leaving a fragment of one gland at its hilum at the first operation. During the second stage procedure, usually performed about 10 days later, the entire remaining adrenal gland was removed. In most patients operated upon during the latter part of the study a bilateral total adrenalectomy was performed

In order to compare the preoperative condition of the patients in both series, the grouping method of Smithwick was used. Table 1 shows that

Table 1. Smithwick Classification of Patients

SMITHWICK GROUP	ADRENALECTOMY-SYMPATHECTOMY PATIENTS	THORACOLUMBAR SYMPATHECTOMY PATIENTS
I	0 (0%)	2 (3%)
II	32 (26%)	19 (25%)
III	34 (27%)	16 (21%)
IV	59 (47%)	39 (51%)

the percentage of patients falling into each of the four groups was closely comparable for both series, a slightly higher percentage (51 per cent) of the patients subjected to thoracolumbar sympathectomy falling into group IV than that of the adrenalectomy-sympathectomy series (47 per cent)

RESULTS

The average preoperative blood pressure level in the adrenalectomy-sympathectomy patients was 209/125 mm. Hg in the supine position The patients surviving their operation sustained an average fall of 51/25 mm Hg, to an average postoperative level of 158/104 mm. Hg. In the thoracolumbar sympathectomy series, the average reduction in blood pressure was 44/27 mm Hg from an average preoperative level of 218/133 mm. Hg. Although the average preoperative blood pressure level in the thoracolumbar sympathectomy group of patients was slightly higher, and the average reduction slightly less than that in the adrenalectomy-sympathectomy group, the blood pressure response was very similar. In both series of patients a further drop was noted in systolic pressure when the patients were in the erect position, this being slightly greater in the thoracolumbar sympathectomy patients

The individual evaluation of postoperative blood pressure responses among the two series of patients is shown in Table 2. If a patient had a blood pressure of less than 150/100 mm. Hg in the supine and standing

Table 2. Postoperative Blood Pressure Response

BLOOD PRESSURE SUPINE AND STANDING	ADRENALECTOMY-SYMPATHECTOMY %	THORACOLUMBAR SYMPATHECTOMY %
Excellent < 150/100 mm Hg	50	30 1
Fair 150/100-185/110 mm Hg	27 4	34
Poor 185/110-200/120 mm Hg	14 3	25 3
Failure > 200/120 mm Hg	8 3	10 6

position, his response was considered "excellent." Fifty per cent of the adrenalectomy-sympathectomy patients had an excellent response as compared to 30.1 per cent of the patients subjected to thoracolumbar sympathectomy. A "fair" response with a postoperative blood pressure ranging from 150/100 to 185/110 mm. Hg was found in 27.4 per cent of the patients in the adrenalectomy-sympathectomy series and in 34 per cent of those in the thoracolumbar sympathectomy group. A larger percentage of "poor responses" and failure were likewise found in the thoracolumbar sympathectomy patients.

Table 3. Postoperative Improvement

	ADRENALECTOMY-SYMPATHECTOMY PATIENTS %	THORACOLUMBAR SYMPATHECTOMY PATIENTS %
Heart size	41.7	38.8
EKG	42 7	32 6
Ocular fundi	55	58

In the follow-up study of these two series of patients, the changes in their electrocardiograms, cardiac size, renal function tests and ocular fundi were followed and recorded. These data were 75 to 90 per cent complete. Improvement in renal function tests was rarely seen in either series. Table 3 shows a comparison of improvement in the electrocardiograms, cardiac size and ocular fundi in the two series of patients. Of the patients with evidence of cardiac enlargement preoperatively as shown by an increase in the transverse diameter of the heart by orthodiagram, 41 per cent showed improvement or a decrease in heart size following adrenalectomy-sympathectomy as compared to 31 8 per cent in the thoracolumbar sympathectomy series. Forty-two per cent of the adrenalectomy-sympathectomy patients had improvement in their electrocardiograms whereas only 32 6 per cent of the thoracolumbar patients showed improvement in their tracings. Improvement in the ocular fundi was found in a slightly higher percentage of patients in the thoracolumbar series (58 per cent) than in the adrenalectomy-sympathectomy series (55 per cent). In the thoracolumbar sympathectomy series a higher percentage of patients showed evidence of progressive impairment or damage in each of these three evaluations than did those in the adrenalectomy-sympathectomy series.

Table 4. Survival Rates

SMITHWICK GROUP	ADRENALECTOMY-SYMPATHECTOMY 0–4 YEARS %	THORACOLUMBAR SYMPATHECTOMY 3–7 YEARS %
I		100
II	91	84
III	80	88
IV	71	54

Table 4 shows the survival rates of the two series of patients. These

figures include an operative mortality of 5 per cent in the adrenalectomy-sympathectomy patients and 1.3 per cent in the thoracolumbar sympathectomy group Because the follow-up period is shorter in the adrenalectomy-sympathectomy series, a comparision of these two series of patients cannot be accurately evaluated. For the length of time these patients have been followed, however, a higher rate of survival has been found in the adrenalectomy-sympathectomy patients in groups II and IV as classified by the Smithwick grouping method.

Despite the higher operative mortality when adrenalectomy-sympathectomy is performed, it has been our impression that the operation carried with it a lower morbidity than thoracolumbar sympathectomy. Postoperative pulmonary complications following thoracolumbar sympathectomy are far more frequently encountered, adding significantly to the patient's period of hospitalization A lower incidence of postoperative complications and an easier anesthetic management have helped make the less desirable operative risk better able to withstand adrenalectomy-sympathectomy than thoracolumbar sympathectomy. With improved methods of adrenal supportive therapy, there are now few problems in the postoperative management of the adrenalectomized patients

SUMMARY

1. Data of 125 patients subjected to adrenalectomy-sympathectomy and followed from 0 to 4 years are compared with data of 76 patients subjected to thoracolumbar sympathectomy and followed from 3 to 7 years.

2 By the Smithwick grouping criteria, the two series of patients were quite similar in the severity of their disease preoperatively.

3 A larger number of patients were found to have a more satisfactory postoperative blood pressure reduction when subjected to adrenalectomy-sympathectomy.

4. A higher percentage of patients were found to have improvements in cardiac size and electrocardiograms in the adrenalectomy-sympathectomy series.

5. Although the operative mortality was higher in the adrenalectomy-sympathectomy series, the postoperative morbidity was less than that in the thoracolumbar sympathectomy series

6 Because of the difference in follow-up period it is difficult to evaluate accurately the two series of patients. For the period of time each series was followed, higher survival rates were found in Smithwick groups II and IV patients treated by adrenalectomy-sympathectomy

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THE EXPERIMENTAL USE OF ORIENTED ELECTRICAL FIELDS TO DELAY AND PREVENT INTRA-VASCULAR THROMBOSIS*

P. N. SAWYER AND B. DEUTCH**

Experimental attempts to use oriented electrical fields to delay and prevent intravascular thrombosis resulted from several previous studies.[1-4] These indicated that the normal blood vessel intima in vivo has a negative electrical potential with respect to the adventitia of the order of at most 1 to 2 millivolts. Evidence accumulated in long term electrode implantation experiments that injury to the blood vascular system or its surrounding tissue led to local abnormalities in the potential difference.[1] Reversal of the measured P D. polarity often occurred. This reversal of P.D. was usually accompanied by intravascular thrombosis.[1] The causal relationships of the two phenomena were studied and indicated that the reversal of potential might act as the trigger mechanism in intravascular occlusion, precipitating out platelets and other formed elements on the injured positively charged vessel wall.

Confirmatory experiments have been conducted using a positively charged field to precipitate a thrombus on the uninjured exposed vessel wall.[2] Polyethylene insulator experiments were carried out in an attempt to delay volume conduction in the damaged tissues and thus selectively delay intravascular thrombosis in damaged blood vessels.[3] These original experiments have been published in greater detail elsewhere and will not be further alluded to here.

Experiments were next designed in an attempt to determine if an oriented field could be used to prevent intravascular thrombosis in acutely damaged segments of blood vessels.[5]

MATERIAL AND METHODS

Normal mongrel dogs, usually young in age, anesthetized with intravenous sodium pentobarbital, were used. Several blood vessels have been utilized in the experiments including the aorta, the paired femoral arteries and veins, the paired carotid arteries and the paired external jugular veins. The vessels are injured by crushing a length of vessel 2 cm. long with five or six mosquito hemostats closed to the second notch and applied for ten

* From the [illegible]

** [illegible] We wish to give grateful thanks to Dr Jonathan E. Rhoads for his help and counsel during the course of these experiments, and to Messrs. David Harshaw and George Dychdala for their many helpful hours in the laboratory

minutes. Then, after removal of the hemostats, a rubber dam is placed between the artery and the surrounding tissues, and the electrodes of choice are applied to the adventitia of both the experimental and the contralateral control artery. The field is created by attaching the battery to the electrode surrounding the experimental artery. The electrode attached to the control blood vessel is not charged The experiment is run for eight to nine hours, following which the animal is sacrificed. The experimental and control vessels are removed by one investigator, examined by another, and compared The grade of thrombosis is classified grade 0 to IV, the larger number signifying an increasing extent of thrombus formation. A zero implies no visible evidence of macroscopic clot. A grade IV thrombosis indicates a complete occlusion of the blood vessel, often a cast of the vessel lumen. The examined vascular segments are placed in 10 per cent Formalin and later sectioned for histologic examination.

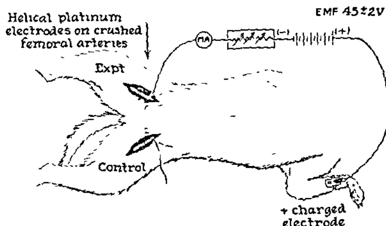


FIG. 1 A schematic diagram of the experimental apparatus showing the electrical circuit The large positive electrode is attached to the dog's left forepaw The negatively charged helical electrode is attached to the crush-injured experimental artery. Note the control vessel, which is injured and is surrounded by an uncharged electrode.

Figure 1 demonstrates the experimental apparatus. It is extremely simple The negative pole of the source of E.M.F. leads to the electrode on the experimental artery through a decade resistance and a milliammeter. The large positive electrode is attached to the forepaw of the dog This arrangement concentrates the negative charges in the injured area of artery and disperses the positive charges. This is done in an effort to prevent positive [illegible] precipitate a thrombus in the [illegible]

The contralateral control artery is injured and has an electrode wrapped around it. However, it acts as a control since no negative charge is applied.

Several types of electrodes have been developed. These are: (1) a platinum foil electrode, which was wrapped around the vessel as tightly as possible. It was made in several sizes, (2) helical platinum wire electrodes and the guard ring wire electrodes, used to create a more even field over the entire surface of the injured vessel and the normal vessel above and below the electrode; (3) a hemicylindrical electrode, designed to cover only one side of a blood vessel; (4) agar-physiologic saline electrodes, designed to

prevent a metal-tissue interface chemical reaction from taking place on the adventitial surface of the artery. An agar-physiologic saline bridge leads from a saline bath to the agar cuff. The metal-physiologic saline chemical reaction is thus established in the bridge water bath and not at the adventitial surface of the blood vessel, as must occur with the platinum electrodes. This would seem to be a more physiologic preparation. However, a high intrinsic resistance is an inherent problem in this type of electrode, limiting the current flow and thus the field density. Chemical reactions must still take place; however, they are of a different nature than occurs with the metal electrodes.

THEORETICAL CONSIDERATIONS

The electrodes were all designed in an attempt to create an electric field of desired shape and thus attempt to prevent intravascular occlusion. The experiments began with the knowledge that the closest possible application of the external field-creating electrode could not anatomically approach the intima-blood interface any more closely than the blood vessel adventitia. It was also recognized that this might be an insuperable barrier to success in this type of experiment.

It was first necessary to demonstrate that a field is actually created by the system which had been established. This was shown by measuring the

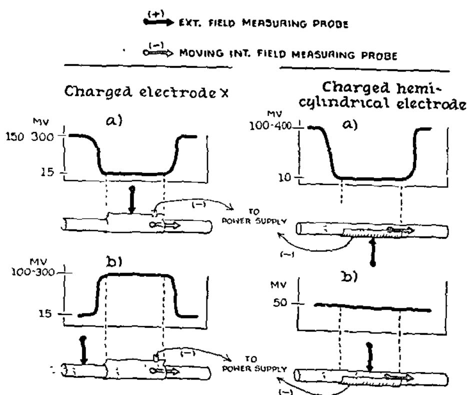


Fig 2 The change in field shape resulting from moving the P.D. measuring probes. On the left the field shape created by the cylindrical electrodes with the external probe in different positions on the ...

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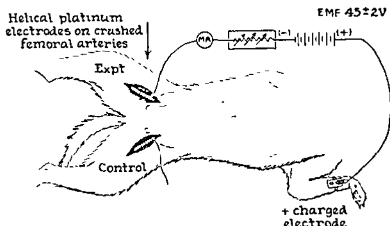


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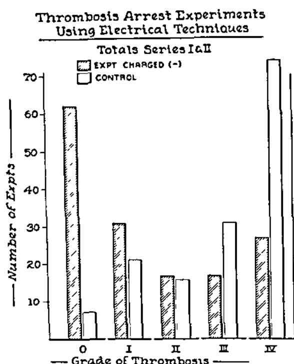


Fig. 3 The results of all the arterial experiments in both series.

DISCUSSION

The experiments would seem to have little practical value in the treatent of prophylaxis of vascular thrombosis. However, the nature of the ɔtting process, according to Quick's theory, is such that once the first atelets are attached to an injured area of blood vessel and break down, e further progress of thrombus formation occurs as an autocatalytic proc-

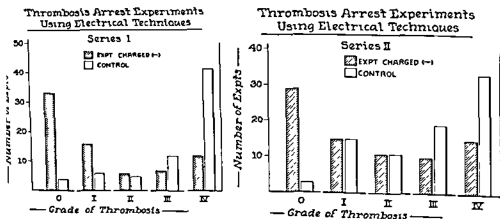


Fig 4 The results of the experiments in series I and II, showing the large number of control vessels which developed an increasing degree of occlusion and the experimental vessels, fewer of which developed a large thrombus.

field established by a hemicylindrical platinum electrode (Fig. 2). If one measures the field on the side of the blood vessel to which the electrode is attached one measures a field similar to that produced by the cylindrical electrodes If one now repeats the procedure on the side of the blood vessel opposite the electrode, no field charge is discernible. This establishes the fact that a field is actually created by the electrode and that the measured P.D.'s are not merely functions of a change in tissue resistance when the probes are moved Determinations of the field shape and magnitude created by each electrode type were conducted They all produced a measured field of similar shape

The examination of the field shape could not be carried out in the same dogs on which experiments in thrombus prevention were being conducted, for the internal measuring probe inserted into the blood vessel as part of the field measuring apparatus acted as a focus for the accumulation of positive charge, increased turbulence in the blood stream, and quickly occluded the blood vessel with a thrombus. These experiments were carried out on separate dogs However, the field created is very stable and easily measurable There is little reason to believe that it varies much from experiment to experiment.

As can be seen in Figure 2, the field falls rapidly toward zero as the internal probe approaches the segment of blood vessel which lies under the current electrode if the external measuring probe lies on the field-creating electrode. Conversely, the measured field reaches a maximum under these conditions if the external measuring probe is placed on the blood vessel at some point away from the field-creating electrode. The decrease in the size of the measured field as the probes approach the center of the electrodes indicates that the thrombosis preventing characteristics of the field will reach a minimum at the center of the electrode.

RESULTS

Two hundred and sixty-eight controlled experiments have been conducted on both arteries and veins, using all of the previously described types of electrodes. In the second series of experiments the blood vessels have been removed by one investigator and examined by another in order to eliminate any bias caused by the knowledge that a vessel was either experimental or control. No particular electrode type prevented thrombosis in the injured vessel in every case. The observed results comparing experimental and control vessel in each [illegible] of the injured arteries in [illegible] large determining factor [illegible] blood vessels which had absolutely no thrombus formation when the contralateral control vessel had a grade IV thrombus. The results of the experiments can be lumped into two series, each of which is divided into subgroups (Figs 3 and 4). In the second series of experiments the observer as well as the experiment was controlled.

Both series of experiments displayed a significant variation between the experimental and the control arteries, indicating that the current probably does have an effect on vascular occlusion.

One additional control series of experiments has been conducted on 26 dogs. In this series the control vessel was positively charged while the experimental vessel was negatively charged. The blood vessels were injured

EFFECTS OF LIGATION OF THE INFERIOR VENA CAVA WITH ABSORBABLE LIGATURE[*]

FAWZI PUALWAN, GLENN E. JONES, STEPHEN J. A. BRUNY, AND W. ANDREW DALE

Ligation of the inferior vena cava for the prevention of pulmonary embolism has proven to be life-saving in selected cases, although at times at the expense of various leg complications secondary to venous hypertension induced by interference with venous return from the lower extremities. The operation is a simple one and has been associated with a low mortality despite its use in patients who frequently are critically ill.

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Previously, the inferior vena cava has been generally tied (or in an occasional instance actually severed) with non-absorbable suture material. Miles and Young[1] reported studies in 34 dogs to learn the best method to insure that once tied, the vena cava would stay tied.

Discussion of a patient exhibiting edematous legs months after caval ligation led to our laboratory study of a method by which the vena cava might be occluded to prevent pulmonary embolism but which would allow later reconstitution of the caval lumen. Ligation with absorbable catgut appeared to be the simplest method

METHODS

A series of 45 dogs has been studied to evaluate the results of absorbable gut ligation of the inferior vena cava. Operations were done under Nembutal anesthesia using aseptic technique. After anesthesia was obtained, venous pressure measurements were made by attaching a saline manometer to a saphenous vein. The zero point of the manometer was always at the level of the table on which the animal rested Further venous pressure determinations were recorded after caval ligation and during the early and late postoperative period (with anesthesia in each instance).

The abdomen was opened, viscera retracted, and the inferior vena cava ligated below the renal veins with a single chromic gut tie pulled up snugly and the knot tied four times. A silver clip was placed on the end of the tie to mark its site by x-ray.

Four types of ligations were done: (1) *High* ligation below the renal veins but above several lumbar veins, (2) *low* ligation just proximal to the caval bifurcation and distal to all lumbar veins, (3) *high plus lumbar* ligation where all lumbar veins distal to the high site of ligation were tied, and (4) *excision* of a part of the cava.

Venograms were made by injection of 20 cc of 70 per cent Urakon into the femoral vein by needle puncture. Later it was learned that these could be made more simply via the saphenous vein.

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ess. Thus the described phenomena may be a trigger mechanism in the production of intravascular occlusion and may have some validity in elucidating the pathologic physiology of intravascular thrombosis. They may also help explain clotting in the cut ends of small vessels during surgery and after trauma.

If some substance could be found which would prevent the physical chemical changes inherent in the damaged intima but which may not be a specific anticoagulant, then the prophylaxis of severe thrombophlebitis or phlebothrombosis might be rendered easier on an ambulatory basis. Phosphorylated hesperidin, recently utilized by Wright and group[7] in anticoagulant experiments, appears to have some of these properties and gives promise of being useful in the treatment and prophylaxis of thrombophlebitis.

SUMMARY

1 The experiments previously conducted by this group in an attempt to explain the etiology of intravascular thrombosis have been alluded to.

2. *Controlled experiments attempting to use oriented fields to delay and* prevent occlusion of previously injured blood vessels have been described. The results of 286 experiments indicate that these fields delay the incidence of intravascular thrombosis under the conditions of these studies. In addition, the tendency of the blood vessels in each animal to thrombose or remain patent *irrespective of whether they were control or experimental* vessels displayed some correlation

3 The significance of the findings is discussed.

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Animals were sacrificed and examined after venograms had indicated deligation of the vena cava. Careful gross inspection was followed by removal of the cava and its main branches. Some of the specimens were used for microscopic sections, while others were digested after filling with Vinylite to make a luminal cast.

RESULTS

Deligation. *Forty-five dogs have been studied to date. Forty dogs had inferior vena caval ligation and 5 had excision of a part of the cava.*

Nine dogs survived high caval ligation and 11 survived low caval ligation for a long period. Seventeen of these 20 (85 per cent) were found by venogram and examination after sacrifice to have deligation with recanalization of the lumen of the cava. Three animals have not shown deligation after 232, 251, and 571 days. Repeated venographic examination and results of the autopsies indicate that reconstitution of the caval lumen occurs gradually, as described by Miles and Young.[1] *The initial tiny opening (or multiple openings) increases in size slowly so that more dye passes at successive venograms.*

It is therefore impossible to state at what exact time the lumens of these cavae became open. We have learned to expect a venographic change at about 100 postoperative days and analysis of data at hand indicates an average deligation time of 117 days. Our data support the findings of Streuter and Paine[4] that there is wide variation in time of deligation of the cava after gut ligation. *However, our series of dogs appears to have shown considerably longer times before deligation.*

A small series (7 dogs) of "high plus lumbar" ligation shows only a single instance of deligation to date. This is associated with development of a larger distal thrombosis by venogram.

Figure 1 shows venograms before and after deligation in a single animal. The photograph of the opened vena cava of the same dog shows the small, white, firmly attached distal thrombus which extended into the iliac veins. *Scarring at the site of caval ligation is apparent.*

Thrombosis Distal to Ligation. Twenty of 40 animals having caval ligation showed either definite venographic or autopsy evidence of thrombosis distal to the site of ligation. Five others showed probable radiographic evidence of such. Eleven of the 15 showing no evidence of thrombosis died in the immediate postoperative period, when it was difficult to differentiate between soft antemortem clots. A high percentage of dogs, therefore, develop *thrombosis distal to the site of caval ligation.*

Venographic and postmortem studies indicate that a soft, dark thrombus forms soon after ligation. Contrary to another report,[1] this has been found to extend beyond the first collateral vein and often to involve the lumbar veins as well as the iliacs and their branches. Its rate of growth is as yet undetermined. Initially this thrombus prevents penetration of dye up to the ligation site, and *shows a coned distal end lying free in the veins. Repeated venograms show increasing dye penetration into the venous lumen, although the walls remain irregular and the lumen narrowed.* Postmortem examination shows a well organized thrombus firmly attached to the venous wall (Figure 1).

In no instance has there been clinical evidence of pulmonary embolism occurring after caval deligation. *The firmly attached, organized thrombosis*

without soft areas indicates little likelihood of embolism from the existing thrombosis. The predisposition to later recurrent thrombosis in such a damaged vein is recognized.[5] Against this possibility must be weighed advantages of temporary caval deligation. The final decision on this question must await clinical trial.

Collateral Venous Circulation. The venogram of Figure 1 shows the extensive collateral circulation which at once develops after caval ligation. Study of over 150 venograms indicates the following points of interest: (1) The vertebral veins appear to function at once as open collateral channels and do not later dilate, while other veins at first appear as small collaterals

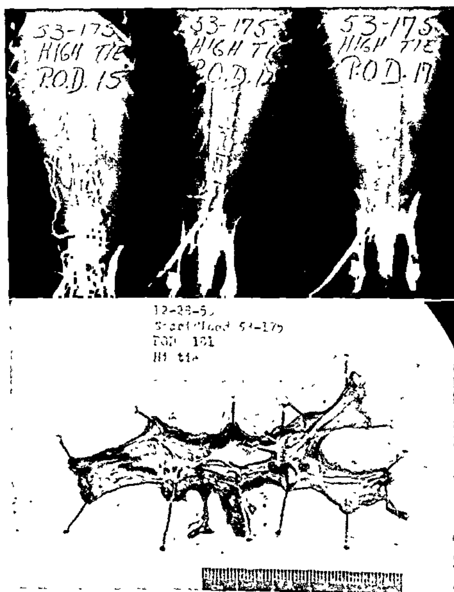


Fig 1. *Above* are venograms on the (left to right) 15th, 121st and 171st day after "high" ligation of the inferior vena cava There is a defect in continuity at the ligation site on the 15th day which is absent on later films Collaterals disappear after the caval lumen is reconstituted *Below* is the opened vena cava and iliac veins after sacrifice on the 181st postoperative day. An organized adherent thrombus is seen in the lumen. White marker (below) shows the site of ligation.

but later become dilated and tortuous (2) There is a change with time from multiple fine collateral veins scattered over a wide area to a few large, *dilated veins lying close to the site of ligation. (3) Satisfactory venograms* of the ligated inferior vena cava can be made by saphenous vein injection. This has been found true in man also

Venous Pressure Following Ligation. Figure 2 indicates the average venous pressure during the postoperative period. Despite a report to the contrary,[3] our findings are similar to those of Moretz, Naisbitt, and Steven-

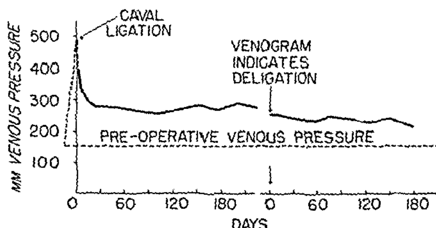


Fig 2. Average saphenous venous pressures of 26 dogs following ligation of the inferior vena cava A second zero time begins the day of the first venogram indicating caval deligation.

son,[2] that after an initial sharp increase, the lower limbs' venous pressure remains considerably higher than the control level for a long time.

It had been hoped that the time of caval deligation would be marked by a sudden decrease of venous pressure. While this occurred occasionally, it was not usual Figure 2, however, does indicate a slow decrease in pressure, and animals are now being studied to learn if this continues.

Deaths Following Caval Ligation. In the course of this study 18 dogs died prior to sacrifice. Four dogs died with distemper clinically. One died at the time of a repeated Urakon injection The remaining 13 deaths were without clinical cause or autopsy finding.

Several of these animals had pitting edema of the lower portion of the body *and legs, lending credit* to the view that these deaths were due to inadequate venous return with pooling of blood in the pelvis and extremities

Table 1 correlates mortality with the site of caval ligation. Deaths are those occurring within 48 hours. Animals dying of distemper later than this

Table 1. Early Mortality (within 48 Hours) by Site of Ligation of the Inferior Vena Cava in Dogs

LIGATION SITE	NO	DEATHS	MORTALITY
High	11	0	0
Low	22	8	36%
High + lumbar	7	2	29%
Excision	5	2	40%

are counted as operative survivals. There was no operative mortality in 11 dogs whose cava was tied "high," i.e., above several lumbar veins. Of the 22 with ligation just above the caval bifurcation (below all lumbar veins) there was 36 per cent mortality. This appears to indicate that the lumbar veins act as important collateral venous drainage after the vena cava has been ligated.

Seven dogs were prepared by "high" ligation of the cava (which should not be dangerous). In addition, all lumbar veins distal to the ligation site were also tied off. Two of these dogs died within 48 hours. A third died at 7 days. This dog is counted as surviving in Table 1, which is perhaps unfair interpretation of the figures. However, addition of lumbar vein ligation appeared to render the high ligation hazardous to the dog.

A series of 5 animals had excision of a portion of the inferior vena cava, thereby reproducing the last discussed series in another way. Two of these dogs died at once.

These deaths therefore indicate that, in dogs, low ligation of the vena cava is more dangerous than is high ligation, since the lumbar veins are rendered useless as collateral channels after low ligation.

Table 2 is an attempt to correlate venous pressure response with mortality. It shows that if the venous pressure returned within a period of 2 hours to a level below 600 mm., the mortality was 16 per cent, whereas if

Table 2. Early Mortality (within 48 Hours) Associated with Lowest Venous Pressure Levels in Immediate Postoperative Period (to 2 Hours)

VENOUS PRESSURE	NO	DEATHS	MORTALITY
0–600 mm	32	5	16%
over 600 mm	6	3	50%

the pressure stayed above 600 mm., 50 per cent mortality occurred. These figures include all types of ligation, although all the deaths occurred following either low ligation or high plus lumbar vein ligation. Deaths occurred with both high and low pressure levels, making prediction of outcome impossible. The high venous pressure animals were more likely to die and this further indicates the importance of collateral venous pathways.

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of ligation in most of these. On the basis of the experience cited we believe that ligation of the inferior vena cava should be done cephalad to several lumbar veins

SUMMARY

The effects of ligation of the inferior vena cava with absorbable gut have been studied in 45 dogs. Eighty-five per cent of operative survivors have shown deligation and reconstitution of the lumen by x-ray and autopsy. Thrombosis was frequently noted distal to the ligation

Ligation of the inferior vena cava caudad to all lumbar veins resulted in an appreciable immediate mortality while ligation cephalad to lumbar veins did not. The venous pressure response to ligations at these different sites indicates the importance of the lumbar veins as collaterals to drain blood from the lower body and legs after caval ligation.

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SPONTANEOUS AND INDUCED CANINE VENOUS COLLATERAL CIRCULATION AFTER CHRONIC EXTRA-HEPATIC OCCLUSION OF THE PORTAL VEIN*

TURLEY FARRAR, JESSE L. BOLLMAN, HOWARD K. GRAY, AND JOHN H. GRINDLAY

Recent reports on the use of some type of portacaval shunting in the surgical treatment of portal hypertension have been encouraging.[3, 11, 14, 19] However, the results in many instances have been equivocal, and not infrequently in cases studied preoperatively and postoperatively by serial roentgen examination and esophagoscopic observation, little or no change has been noted in the size of esophageal varices. Learmonth[13] in 1949 reported a case in which death occurred 4 days after a splenorenal shunt done for recurrent episodes of bleeding from esophageal varices, and in this case a large natural shunt from the splenic vein to the left renal was found at necropsy. This vein was about 6 mm. in diameter and yet it had not prevented bleeding from esophageal varices. Snell[22] in 1950 in a panel discussion of the portal circulation expressed some doubt as to the effectiveness of limited shunting procedures in the surgical treatment of portal hypertension. Beswick and Butler[2] reported a case of fatal hematemesis from esophageal varices in the presence of a large portacaval anastomosis.

The more recent introduction of other surgical procedures in the treatment of portal hypertension, such as ligation of the hepatic artery recommended by Rienhoff[18] and Berman and Hull,[1] and the direct transesophageal obliteration of the varices reported by Boerema[4] and Crile,[7] attests to the existing confusion regarding surgical treatment of this condition. Direct measurement of portal venous pressure in man has been of very little aid in clarifying this condition. Gray[9] reported a wide variation of intraportal pressures in man when no evidence of hepatic disease or extrahepatic portal disease was present. Palmer[16] found no great correlation between portal venous pressures and the severity of esophageal varices among different

* [illegible] Graduate School [illegible] the degree of [illegible]

patients. The confusion arises in part from several poorly understood phenomena accompanying portal hypertension, one of which may be the naturally occurring portacaval shunts.

The present study of natural portacaval shunts occurring in portal obstruction was undertaken to determine the relative importance of the various collateral pathways with emphasis on the role of esophageal veins, and the development of venous channels in omental adhesions.

METHODS AND MATERIAL

Healthy mongrel dogs were used. All dogs were prepared by placing a laminated cellophane band around the portal vein after the method de-

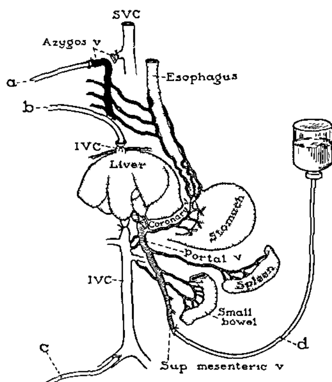


Fig 1. Perfusion system *a*, outflow from azygos vein, *b*, outflow from hepatic vein, *c*, outflow from abdominal inferior vena cava, and *d*, inflow cannula.

scribed by Hoffbauer, Bollman and Grindlay.[10] This procedure produces *gradual occlusion of the portal vein*

After periods ranging from 2 to 3 months, surgical procedures to shunt portal venous blood both to the liver and to the caval system were carried out. The following procedures were used.

Group A Procedure. Through an upper midline abdominal incision the spleen was removed but the main splenic vein was preserved. The left portal vein at the hilus of the liver was identified (cephalad to the previously applied cellophane band). A curved brass probe with an eye in the end was inserted into this vein, guided up into the left lateral lobe of the liver and thrust through the parenchyma of the liver to the under surface of this lobe.

A silk suture was passed through the open end of the preserved splenic

vein and threaded through the eye of the brass probe. The probe was then withdrawn, pulling the splenic vein up into the intrahepatic portal vein It was held in place by suturing the silk draw thread to the wall of the portal vein where the brass probe entered Small absorbable gelatin sponge (Gelfoam) strips adequately controlled the slight amount of venous bleeding at the site of implantation of the splenic vein into the liver.

There were 10 dogs in the group.

Group B Procedure. Through a right thoracicoabdominal incision in the tenth intercostal space the peritoneum over the upper pole of the right kidney and the right adrenal was excised The surface of the right lateral lobe

Table 1 Portal-Perfusion Flow

GROUP	DOG	TIME AFTER PORTAL LIGATION, DAYS	WEIGHT, KG	FLOW PER 4-MINUTE PERIOD, CC.* AZYGOS	ABDOMINAL INFERIOR VENA CAVA	HEPATIC
A	1	139	8 7	9	282	38
	2	139	9 2	165	168	8
	3	145	10 9	245	178	68
	4	146	17 6	42	540	6
	5	146	11.7	65	430	3
	6	153	17.6	61	312	22
	7	138	8 6	170	283	48
	8	152	13 2	145	325	22
	9	151	11 7	100	178	46
	10	134	7.0	38	468	5
B	11	99	7 0	66	96	10
	12	112	4 6	86	240	18
	13	342	13 9	82	223	19
	14	354	13 6	112	274	20
C†	15	243	7 5	30	358	12
	16	277	15 5	298	332	14
	17	275	8 0	174	288	14
	18	266	9 2	268	176	8
	19	235	16 0	199	148	18
Normal	20			0	0	344
	21			0	8	272
	22			0	42	195
Eck-fistula	23			3	465	7

* The average of two or more 4-minute periods
† Perfusion studies were done in 5 of the total of 9 animals in this group

of the liver was scarified and this lobe of the liver was sutured to the denuded area of the kidney and retroperitoneal tissue.

There were 4 dogs in this group

Group C Procedure. Two types of omentopexy were done. In type 1 the superior surface of the left lateral lobe of the liver was scarified and the omentum was fixed to this area with multiple fine catgut sutures In type 2 the peritoneum was resected from over the upper pole of the right kidney and from the right posterior abdominal wall, exposing the retroperitoneal area along the inferior vena cava The free omentum was then sutured to this denuded retroperitoneal region with fine catgut sutures.

There were 9 dogs in this group.

After a waiting period of 2 to 5 months from the time of the last surgical procedure, the dogs were killed with an overdose of pentobarbital sodium (Nembutal) given intravenously, and after the chest and abdomen were opened perfusion studies were set up in the following manner (Fig. 1).

The azygos vein was divided at its entrance to the superior vena cava, and the distal end was cannulated. This cannula is labelled *a* and represents flow through the esophageal veins. The inferior vena cava was divided just above the diaphragm, the distal end was cannulated and the other end was



Fig 2 Dilated, tortuous, periesophageal and esophageal veins. *A.V*, Azygos vein, *E*, esophagus, *S*, stomach

ligated. This cannula is labelled *b* and represents flow through the hepatic vein

The inferior vena cava was also ligated just below the diaphragm. The right common iliac vein was cannulated just below the bifurcation. This cannula is labelled *c* and represents all collateral flow from the portal to the caval system below the diaphragm, except possible small collaterals which might ent

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A plastic catheter (No. 13) was inserted into a major branch of the superior mesenteric vein and by means of an intravenous set the perfusate

was administered through this catheter. Warm water (temperature about 110°F) was used as the perfusing fluid with the intravenous bottle elevated 95 cm above the level of the portal bed. At this level 700 cc. of warm water flows through the unobstructed catheter in a 4-minute period.

After thorough flushing out of the portal system and its collaterals the perfusate from each cannula was collected in separate containers, measured and recorded. A 4-minute period of flow was arbitrarily chosen as adequate time for determining relative rates of flow. The average of two or more 4-minute periods of flow was obtained and recorded (Table 1).

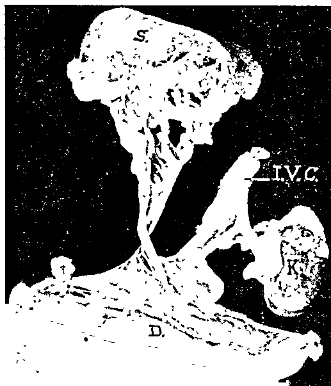


Fig 3 Collateral vein coursing from the hilus of the spleen to the left renal vein *K*, Left kidney, *S*, spleen, *D*, duodenum, *I V C*, inferior vena cava

After perfusion studies, a barium-gelatin mixture was injected into the portal system through the same catheter. This injection medium, after it gels, greatly facilitates dissection of the veins.

RESULTS

There was variation in size and number of the various portacaval collaterals but in general the following pattern was revealed.

Periesophageal Veins. The two longitudinal periesophageal veins were dilated and tortuous in every animal. In a few dogs the posteromedial vein was 5 to 6 mm in diameter and the anterolateral vein was dilated up to 4 mm in diameter (Fig 2).

Hepatoduodenal Veins. One to three veins ranging from 1 to 2 mm. in diameter were found coursing through the hepatoduodenal ligament. These veins were noted in every animal and emptied into the portal veins at the hilus of the liver, proximal to the portal constriction.

Retroduodenal Veins. The presence of numerous small retroduodenal veins ranging from less than 1 mm. up to 4 mm. in diameter and running from the portal system to the inferior vena cava, left phrenicoabdominal vein and left renal vein was a constant finding.

Splenorenal Veins. A constant finding was a vein, from 3 to 6 mm. in diameter, coursing from the hilus of the spleen to the left renal vein (Fig. 3).

Omental Veins in Adhesions. In the majority of animals venous shunts were found in postoperative omental adhesions to the anterior abdominal wall at the site of upper abdominal incision. These veins ranged from less than 1 mm. up to 4 mm. in diameter and varied in number from five up to

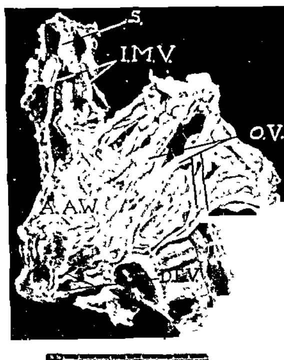


Fig 4 Portacaval shunts by way of omental veins anastomosing with systemic veins of the anterior abdominal wall S, Sternum, *I M V*, internal mammary veins, *D E V*, deep epigastric veins, *A A W*, anterior abdominal wall, *O V*, omental veins.

ten. Continuity between the omental veins and the epigastric veins of the anterior abdominal wall was readily demonstrated (Fig 4).

Miscellaneous Veins. Retrocolic veins (1 to 2 mm. in diameter) emptying directly into the inferior vena cava were occasionally noted. In 3 animals a vein running from the duodenal mesentery to the right renal vein was found

Venous connections between the superior hemorrhoidal vein on the one hand and the hemorrhoidal veins of the caval system on the other hand were noted in a few dogs but were rather small.

Animals in group A, which had implantation of the splenic vein into the intrahepatic portal vein, all had stenosis of the splenic vein at the site of implantation. Even though the splenic vein remained patent to the point where it entered the liver, in only 1 dog was there evidence of an open anastomosis, although the lumen at this point was very small, being of pinpoint size.

Animals in group B had much scarring in the region where the right lateral lobe of the liver and the kidney had been sutured together. However, medium injected into the intrahepatic portal system was demonstrated in the renal parenchyma and the retroperitoneal tissue above the kidney. Numerous small thread-sized veins (venules) were noted running through the adhesions, but no large communicating veins were demonstrable

Four animals in group C had omentum sutured to the retroperitoneal structures as described. Only 1 of these had communicating veins between omentum and the retroperitoneal veins. In the other animals no omental adhesions were noted; apparently the sutures holding the omentum in place had cut through.

Animals having suture of omentum to the liver showed fine communicating veins (the size of No. 30 sewing thread). There were six to ten of these per square centimeter of surface area of omental adhesions. No large communicating veins were demonstrated.

The venous pattern of the lower part of the esophagus varied little in the different groups, consisting of two longitudinal periesophageal veins and two longitudinal submucosal veins with a few small connecting veins between them About 6 cm. above the cardia, these periesophageal veins emptied into the azygos vein by means of two or more branches. By way of comparison, Kegaries[12] demonstrated three to four longitudinal periesophageal veins and the same number of submucosal veins in the lower part of

veins

ly the

larger and frequently appeared to be a direct continuation of the gastric coronary vein.

The submucosal esophageal veins were dilated very little if at all.

COMMENT

It is recognized that portal hypertension comparable to that found in man does not occur in dogs after gradual obstruction of the portal vein with a cellophane band Hoffbauer, Bollman and Grindlay[10] found the average increase in portal pressure in the dog following this type of obstruction to be about 7 cm. of water, producing a portal pressure of approximately twice the normal reading This increase of the pressure gradient between the portal and the caval systems, even though small, may be sufficient stimulus to produce large collateral shunts

Development of natural portacaval shunts following prolonged ligation of the portal vein with a cellophane band has been mentioned previously by Volwiler, Grindlay and Bollman,[24] and these shunts are strikingly similar to those that develop in man when the portal vein is obstructed Such shunts found in man actually represent a dilatation of normally occurring small veins that connect the portal with the caval system and have been described by Retzius,[17] Sappey[20] and Charpy[6]

Morris and Miller[15] in an experimental study of the collateral circulation

The results of perfusion-flow studies in our experiment would indicate that the coronary-esophageal-azygos flow is quite significant in dogs with occlusion of the portal vein below the hilus of the liver.

We found no large connecting veins in induced adhesions of the omentum to the liver; however, numerous small thread-sized connecting veins were demonstrated.

The constant finding of large collateral channels to the left renal vein was most striking. Similar findings have been reported in man; Simonds,[21] Learmonth,[13] Beswick and Butler,[2] and Edwards[9] have cited individual cases in which large collateral veins ran from the splenic vein or some other branch of the portal system to the left renal.

This fact might be of clinical significance since the surgeon, unsuspectingly, may obliterate important collateral veins in mobilizing the left renal in preparation for a splenorenal-shunt procedure.

Brunschwig and co-workers[5] did not find venous connections between omental veins and veins of the anterior abdominal wall in omental adhesions that they induced in animals without partial obstruction of the portal vein. They observed that dogs having had this type of omentopexy 3 weeks prior to acute occlusion of the portal vein all died, and concluded that omental adhesions were of no value in so far as affording portacaval shunting is concerned. In our study portal constrictions were established prior to omentopexy.

We found small connecting veins at the site of omentopexy as early as 4 weeks after application of the cellophane band to the portal vein. Very large omental venous collaterals were found in a few dogs after periods ranging from 2 to 3 months.

CONCLUSIONS

On the basis of the observations made in this study, the following conclusions seem warranted:

1. Numerous and large collateral veins develop between the portal and the systemic venous system after gradual occlusion of the portal vein.
2. These pathways parallel those found in man with portal obstruction.
3. The coronary-esophageal shunt may account for as much as 50 per cent of the total (shunt flow) as measured by portal-perfusion studies.
4. The periesophageal veins as a rule are markedly dilated and tortuous. The intramural esophageal veins appear normal or only slightly dilated.
5. No submucosal esophageal varices are found.
6. The large and frequently multiple venous connections between the portal system and the left renal are a constant and significant finding.
7. Patent venous shunts can be demonstrated at the sites of spontaneous and induced omentopexy.

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THE USE OF RADIOACTIVE SODIUM IN THE DETERMINATION OF PATENCY OF PORTACAVAL SHUNTS*

RALPH A. DETERLING, JR., SAMUEL R. POWERS, AND
SHIVAJI B. BHONSLAY**

The portacaval shunt has been shown to be an effective measure in significantly reducing [illegible] esophageal and gastric varices in patients with [illegible] protection depends on the continued patency [illegible] between the portal and caval venous systems, through which decompression of the portal bed is possible. It is frequently of clinical importance to determine whether a shunt has remained open, and the variety of measures which have been tried indicates that none have been entirely satisfactory.

Among the simpler methods have been the direct correlation of the status of esophageal varices to level of portal venous pressure. Blakemore[1] and others have noted the marked reduction in size of varices demonstrable by esophagram following production of a satisfactory portacaval shunt, and have correlated this finding to reduction in portal pressures observed at operation and to absence of subsequent bleeding. Unfortunately, not all patients with portal hypertension and treated for bleeding at Presbyterian Hospital revealed significant varices by esophagram. Furthermore, the size and distribution of varices shown by esophagram do not bear a critical relationship to actual portal pressures found at operation.[4]

Palmer has advocated reliance on esophagoscopy for the diagnosis of varices because of failures by esophagram, and has developed a technique for measurement of pressures in the varices at time of endoscopy.[5] In a study of patients who had had shunt operations, he noted a significant reduction in pressure in varices following surgery, but he denied a close correlation of any single level of portal pressure or of specific changes in portal pressure to actual severity of varices.[6] If the radiologic pattern of esophageal varices is merely a variable element in the collateral formation in the individual patient rather than a predictable and consistent indicator of elevated portal pressure, its changes cannot always be considered as reliable evidence of patency in patients, especially in those with questionable esophageal varices present before operation. There has been reluctance by some to employ endoscopy routinely in patients with esophageal varices, especially if needle puncture of a varix is to be performed. A fatal hemorrhage has occurred in at least one such patient.[7]

Other tests have been based upon visualization of the actual anastomosis by injection of an iodide preparation through a catheter introduced into the inferior vena cava[8] or portal vein.[9] More recently, visualization of the portal vein has been effected by injection of contrast medium percutaneously into the pulp of the spleen.[10-12] We have made use of this method in the post-

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operative period to demonstrate patency of a shunt in patients from whom the spleen had not been removed. However, this test is impossible in those in whom splenectomy with splenorenal shunt has been performed.

The pressure alterations in the portal system observed through measurements from a catheter left in the portal vein via a mesenteric branch at time of surgery[13] or from one manipulated into the portal vein[9] or left renal vein[14] at some time following surgery have been guides as to patency of a shunt. In a patient reported by Leger and associates, an elevation of pressure in the left renal vein was evidence of an open shunt.[14] They also employed test substances deposited in the duodenum by tube including bile salts or glucose, the concentration of which was compared in blood from the renal vein, inferior vena cava and from a peripheral vein. Efforts of a similar type had been carried out by Bradley and associates[15] with glucose, galactose and other substances in 1948 and subsequently by Weissman[16] at Presbyterian Hospital without consistent or significant differences. Allard, Harpur and Johnson[17] described the technique and reported success in one patient with a splenorenal shunt.

In 1948 Deterling and Quimby[18] studied the appearance time and concentration curve of radioactive sodium in the foot after ingestion of the test substance in water and various concentrations of sodium chloride In a series of normal and cirrhotic patients, before and after portacaval shunt, there was such variability that the method was considered unsuited for determination of patency of a portacaval shunt. De Almeida[19] instilled 1 ml. of ether in 5 ml. of serum into the third portion of the duodenum by tube, and noted that the time of appearance in the lungs of normal individuals was about 12 seconds The test was modified by Zérolo[20] in that saccharin was substituted and a more definite end point was achieved by taste Normal values by this means ranged from 45 to 50 seconds, and from 110 to 195 seconds in cirrhotic patients. Only a very few patients were described in the report, however [14]

In 1949, Newman and Cohen[21] reported on the time of transit of ether vapor instilled into the rectum until recognized in expired air from the lungs. This ranged from 11 to 25 seconds in 34 normal patients, and in 7 cirrhotic cases was increased to a range of 34 to 180 seconds Fourteen other patients with varying hepatic disorders were also studied. They concluded that the retarded passage of test substance indicated decreased velocity of portal blood flow, in response to increased resistance in the liver. However, similar studies by Brugel[22] and Ragab[23] failed to confirm these conclusions.
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latter report surveyed the results of 161 tests in 86 patients There was wide variation in the transit time obtained in the entire group without shunts, and it appeared that such a test indeed did not give conclusive indication as to the liver function or level of portal pressure However, it was noted that a consistent reduction in transit time was obtained in patients in whom patency of a portacaval shunt was confirmed by finding a reduced pressure in the esophageal varices, as per the method of Palmer There were definite practical objections to the test in that a large volume of vaporized ether had to be injected as rapidly as possible into the rectum, and the first trace of ether from the lungs detected by the patient and two observers

Believing that there might be value to the principle involved, we tested seve ... the shunt opei ... sscein into the ... or signs of fluorescence. The end point was too vague to give reliable values, however. Subsequent studies, employing a testing solution of radioactive sodium, form the basis for this report.

METHOD

A total of 64 tests were performed on 24 patients with portal hypertension and 10 patients without liver disease. A shunt was created in 16 patients. In many instances multiple tests were performed before and/or after crea-



Fig. 1 The time interval between the injection of Na^{24} into the rectum by assistant (at left) and appearance in brachial artery is measured by stopwatch as well as by continuous ink-writing recording apparatus

tion of a portacaval or splenorenal shunt. In one patient a shunt was effected between a minor tributary of the portal system and the vena cava.

The bowel is prepared by colon lavage shortly before the test. A moderately stiff 20 French rubber catheter 16 inches in length is inserted its full length into the rectum. If there is question as to position or looping, the catheter should be advanced under fluoroscopic control. The patient is supine or in slight Trendelenburg position during the test period. The abducted left arm of the individual is shielded with lead bricks, and the trunk is also shielded by means of a leaded rubber apron or sheet lead. A volume of 20 to 25 ml. of saline containing an average test dose of 100 to 150 microcuries of Na^{24} is injected rapidly by bulb syringe after which the catheter is clamped. The count is effected by means of an end window Geiger tube with a 1.3 mg./cm.^{2} window, overlying the brachial artery. The counts were measured on a count rate monitor* and read directly or recorded continu-

* Model 410, Atomic Instrument Co., Cambridge, Massachusetts

ously on a direct writing apparatus* (Fig. 1). The test material is distributed in the rectosigmoid area, and is absorbed rapidly into the splanchnic veins (Fig. 2) A sharp and distinct rise in the count well above the background values indicates the appearance time in the brachial artery (Fig. 3).

In a series of 18 adult dogs, with and without side-to-side portacaval shunts, confirmatory tests were performed In certain animals, counts were obtained directly from blood passing through a special chamber, from the inferior vena cava or carotid artery. A dose of 60 microcuries of Na^{24} was injected either into the spleen or bowel, before and after creation of a side-to-side portacaval shunt. In all instances, there was a marked decrease in



Fig 2 Injection of 10 ml of saline containing 150 μc of Na^{24} and 15 ml of 30 per cent Urokon into rectum, with patient in 7 degrees of Trendelenburg position Record of this test revealed patency of a portacaval shunt (see Fig 3)

transit time following the opening of the shunt, from values above 35 seconds to those below 20 seconds.

RESULTS

An analysis of the records obtained from the control and the pre-shunt patients with portal hypertension reveals a great variation in transit time of the Na^{24} from rectum to arm Values ranged from 55 to 130 seconds (Fig. 4). As observed by others,[22-25] there was no consistent correlation to degree of liver dysfunction or portal pressure measured at time of operation On the other hand, following the production of a portacaval or splenorenal shunt, [illegible] with open [illegible] 25 to 30 [illegible] ient with [illegible] lue of 45 seconds was observed. It was impossible to confirm patency by other means in this patient, but in any event it was a relatively ineffective shunt. In three

* Brown Electronik, Honeywell, Minneapolis, Minnesota.

patients, the status of the shunt was ascertained at autopsy, and confirmed the test (Fig. 4). Two of them had the onset of severe cholemia following shunt and serious bleeding from the colon occurred in one of these. The clinical impression *strongly favored closure of the shunt as contributing to* the patients' course. In both, the transit time was less than 30 seconds and at autopsy shortly thereafter, completely open anastomoses were found. The third patient did poorly following a splenorenal shunt and at autopsy was found to have an occluded anastomosis The pre-shunt value was 110

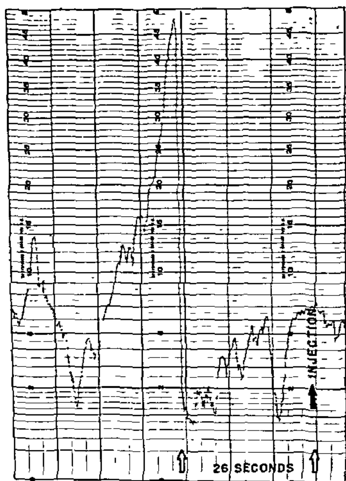


Fig 3 Injection of 150 μc is marked at right and an abrupt rise in count above background levels was observed at 26 seconds A repeat test several days later gave the same value. Patency of the portacaval shunt in this patient was confirmed by pre- and postoperative esophagrams and transsplenic portograms

seconds and prior to death it was 160 seconds. Six patients were readmitted for study at some time after a shunt had been created; the test revealed a time in excess of 65 seconds in two patients Although no test had been obtained before operation, the indication that the splenorenal shunts had closed was supported by bleeding in one and large esophageal varices in both. In the others, all with *portacaval shunts*, the short transit time confirmed the clinical impression supported by esophagram that the shunts were functioning In other instances, patency was *suggested* by a marked reduction in transit time as compared with pre-shunt values Confirmation

was obtained by esophagram or visualization via the spleen. Repeat tests with Na^{24} at intervals of a few days to several weeks revealed a consistency in most of the pre-shunt group, and in all of our post-shunt patients with patent anastomoses

DISCUSSION

The review of tests of patency recorded in the literature indicates certain faults in each. Some were inconsistent or had vague end points Others were

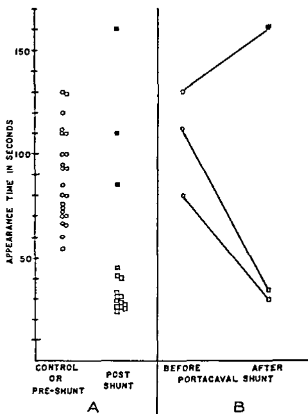


Fig 4 *A*, Representative values obtained by the isotopes test The transit time varied between 55 and 130 seconds in normal cases or patients with portal hypertension Following a shunt operation, much lower values were observed, except in cases with occluded shunts (black squares)

B, Status of shunt as predicted by the test was confirmed by autopsy in three patients In two, the anastomosis was open as suggested by a short transit time In one, the splenorenal anastomosis had thrombosed, as had been predicted by the test (black square)

applicable only to certain patients with portal hypertension, as for example, esophagram or transsplenic visualization. Possible risk has restricted the use of the latter method in the postoperative period Catheterization of the portal or left renal vein entails a major procedure in which experience and dexterity are required.

A completely satisfactory test should have certain qualifications which seem to be met by the use of isotopes (*a*) applicability to all patients, (*b*) simplicity as a method, (*c*) use of commercial equipment, (*d*) use of well tolerated but foreign substance with a sharp end point, (*e*) consistent

and reliable results, (*f*) safety. The average body dose of irradiation was equivalent to less than 0.2 r, or well below permissible limits set by the International Commission on Radiological Protection.

The specific factors involved in the determination of circulation time in the intact splanchnic and hepatic beds are very complex and include rate of blood flow, blood volume, length of the shortest circuit, configuration and vasomotor alterations of the bed during study. These must be considered separately and require quantitative techniques.[26] The meaning of the transit time as measured with Na^{24} is not clear except that an alteration appears to occur with an open portacaval shunt. Tentative inferences may be drawn regarding patency, but final conclusions must await accumulation of sufficient quantitative data. The variability in normal and pre-shunt patients with portal hypertension as observed in this series and that of others[22-25] obviously precludes any interpretation in respect to splanchnic flow, portal pressure or hepatic function.

On the other hand, if we accept values obtained for systemic circulation time as valid, the differences in our post-shunt time and systemic circulation time (vena cava to arm) should represent transit time from mucosa of bowel to vena cava via the shunt. Since the systemic circulation time varies between 14 and 24 seconds (average 18 seconds) it is evident that bowel to cava time via a shunt is a matter of a few seconds. This was confirmed by our experiments in dogs studied with the open abdomen. Hunt[27] has performed supporting studies with Na^{24} injected into portal tributaries at operation, and transit time over a 7 5 cm. distance recorded by a bipolar scintillometer The normal patient had a velocity of 10 cm per second. After portacaval shunt, the average speed was 12 cm per second, and after splenorenal shunt the average speed was 7 2 cm. per second. The slight increase in our post-shunt bowel to arm value over systemic circulation time would be compatible with these studies on velocity of portal flow. The difference also supports the assumption that absorption occurs at levels above the inferior hemorrhoidal system, and consequently does measure transit time in the splanchnic veins It is our belief that this test is of value to determination of patency of an effective shunt between the portal and caval systems.

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A METHOD FOR DETERMINING THE PATENCY OF A PORTACAVAL SHUNT*

B EISEMAN, G. LINDEMAN, AND JOAN JOHNSON

There is no simple means now available for determining the postoperative patency of a portacaval shunt The purpose of this paper is to describe such a chemical method which is based on the difference between the oral ammonium citrate tolerance curve when portal blood by-passes the liver in contrast to the curve with normal liver perfusion

Ammonium citrate when taken by mouth is absorbed from the small intestine and is transported via the portal vein to the liver where the ammonia radical is converted into urea Normally, blood ammonia levels do not rise appreciably following ammonium salt ingestion. Even far advanced cirrhotics can synthesize urea from ammonia so long as the hepatic cells are properly vascularized [1, 6]

While studying blood ammonia levels in cirrhotic patients and in animals following portacaval anastomoses, we found that ingested ammonium citrate produced prolonged and markedly elevated blood levels where a portacaval

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shunt existed. These findings, which had been noted by others,[1, 4, 6] were utilized to form the basis of the present test of vascular patency.

MATERIALS AND METHODS

Side to side portacaval anastomoses were carried out on 6 fasting adult mongrel dogs using a right thoraco-abdominal incision When the anastomosis was completed 7 grams of ammonium citrate dissolved in 20 ml. of tap water were introduced into the proximal jejunum by means of an indwelling intestinal tube. Peripheral venous blood was withdrawn from an extremity vein prior to the installation of the ammonium citrate and at 30 minute intervals thereafter until blood ammonia levels were normal.

Blood ammonia levels were determined by the method of Seligson and Hirahara,[9] which is based on measurement of ammonia liberated from a buffered solution by diffusion.

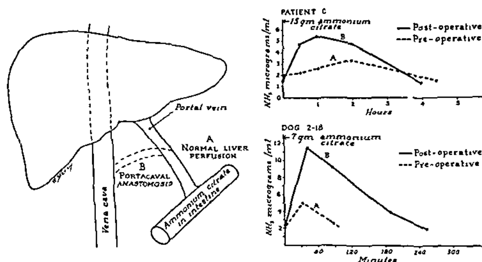


Fig 1 Oral ammonium citrate tolerance curves of human and dog, (*A*) with normal liver perfusion, (*B*) after producing a portacaval vascular shunt

By placing an occluding vascular bulldog clamp between the shunt and the hilus of the liver (Fig. 1), all of the portal blood was diverted into the vena cava A return to normal portal liver perfusion was obtained by occluding the shunt and removing the bulldog clamp from the portal vein. We variously occluded the shunt by using a Potts-Smith clamp on the vena cava, or obliterating the anastomosis with arterial silk. Comparison of ammonia tolerance curves with and without a vascular hepatic by-pass could thus be obtained

In order to have more perfect control studies ammonia tolerance curves were performed first with the shunt open and then occluded, as well as reversing the sequence of study. This obviated the criticism that the later experiments were carried out on animals whose liver function was altered by prolonged operation, anesthesia, or by vascular shunting

The end to side anastomoses in dogs were technically easier, better tolerated by the animals, and were better adapted to our plan of study. When re-establishment of normal flow through the liver was desired, the shunt

was taken down and an end to end anastomosis performed upon the interrupted segments of the portal vein.

RESULTS OF ANIMAL EXPERIMENTS

Peripheral blood ammonia levels increased but slightly following the installation of ammonium citrate into the intestinal tract of animals in which portal blood was perfused through the liver. Such an ammonium tolerance curve is illustrated in Figure 1A and is characterized by a peak of less than 5 micrograms per milliliter and a return to normal levels within a period of 2 hours.

In the presence of a patent portacaval shunt with the portal blood bypassing the liver, peripheral blood ammonia concentrations mirror the amounts of ammonia being absorbed from the intestinal tract. The maximum concentrations appear within the first hour and reach a higher peak and



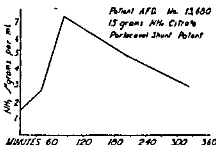


Fig 2. Correlation between splenoportogram and ammonium citrate tolerance curve in a patient with a patent portacaval anastomosis

remain elevated for a more prolonged period than is the case with the occluded shunt (Fig. 1B)

Reversing the operative sequence resulted in similar ammonium tolerance curves, the closed shunt producing a low flat curve despite the previous prolonged vascular shunt.

CLINICAL STUDIES

The patency of portacaval anastomoses has been studied according to this technique in a series of 8 patients Preoperative ammonium studies have been performed in 3 of these patients (Fig. 1, patient C). In three other cases, the spleen was available for splenoportograms[2] and visualization of the patent shunt could be correlated with our chemical studies (Fig. 2).

In all cases the blood ammonia concentrations have reached higher levels and have remained elevated for more prolonged periods in the presence of a patent portacaval shunt than was the case preoperatively. The maximum concentration found in this series was 8 1 micrograms per milliliter, and the

longest period of elevation above normal was 6½ hours. These figures depend, of course, on the dose of ammonium citrate ingested and its rate of absorption.

In one case the ammonium citrate tolerance curve indicated occlusion of the anastomosis, a fact confirmed by splenoportography (Fig. 3) and at the time of subsequent laparotomy.

Eight unoperated patients with varying degrees of cirrhosis have been studied according to this technique. Our findings agree with those of Caulaert,[1] who in 1932 found that peripheral ammonia blood levels rose

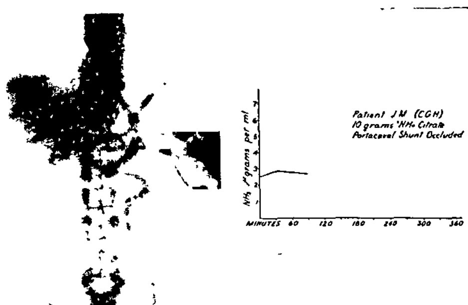


Fig 3 Correlation between splenoportogram and ammonium citrate tolerance curve in a patient with an occluded portacaval anastomosis.

only slightly under these circumstances. In no case did they approach the concentrations found when a patent shunt served to by-pass the liver.

DISCUSSION

A major clinical need exists for a reliable chemical method for determining the patency of a portacaval shunt Without it, the clinician is never certain that the shunt so obviously patent at surgery has not occluded thereafter. When the spleen has not been removed it is possible to visualize the shunt by splenoportography,[2] but unfortunately this method carries a certain risk, and the spleen is not uncommonly surgically absent in this group of patients Measurement of the portal circulation time has been employed, but is both difficult to perform and inaccurate in interpretation.[5]

Certain factors undoubtedly alter the absolute accuracy of this test Liver damage per se apparently does not alter the ability of the hepatic cells to convert ammonia to urea and so to clear the peripheral blood of this substance.[1] In some cases of cirrhosis, however, small intrahepatic shunts exist that by-pass active hepatic cells and may alter the ammonia-urea conversion. Extrahepatic channels by-passing the liver, such as esophageal varices, will also undoubtedly produce elevations in peripheral ammonia levels following ammonium citrate ingestion. Only by performing preoperative am-

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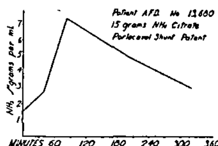


Fig. 2 Correlation between splenoportogram and ammonium citrate tolerance curve in a patient with a patent portacaval anastomosis.

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AN EVALUATION OF AMMONIA INTOXICATION IN NORMAL DOGS AND IN DOGS HAVING A PORTACAVAL ANASTOMOSIS*

ROBERT H. DE RIEMER, DONALD E. HINE, AND HAROLD A. HARPER

Hahn, in Pavlov's laboratory,[1] described experiments on dogs with a portacaval anastomosis in which staggering gait, bizarre reflex changes and sometimes coma or death occurred after a short period of feedings of raw meat. The syndrome was associated with elevated levels of ammonia in the blood. Similar symptoms have been induced by the administration of ammonium salts.[5] Increased blood ammonia and similar central nervous system disturbances also have been described in patients with so-called "hepatic coma,"[7] and in others without liver disease in whom portacaval anastomoses have been done.[6]

Glutamic acid and its keto acid precursor, ketoglutaric acid, are known to accept ammonia readily, and it is presumed that such reactions as well as urea formation are important methods for the detoxification of ammonia by the liver. Clinical studies on the effectiveness of these substances in the treatment of ammonia intoxication have given varying results.[8, 10]

The following experiments were carried out to test the therapeutic effects of glutamic acid, ketoglutaric acid and sodium succinate in dogs with acute, artificially induced ammonia intoxication, as well as in those with the syndrome of "meat intoxication." The changes occurring in the *p*H, ammonia, and urea nitrogen of the blood, as well as in the plasma glutamine, glutamic and aspartic acids following the administration of these compounds, were also measured

All determinations were made on blood drawn in a heparinized syringe. The blood *p*H was measured electrometrically. Blood urea nitrogen was determined by the method of Van Slyke and Kugel.[9] Blood "ammonia" determinations were done according to Conway.[2] The glutamine, glutamic acid and aspartic acid concentrations in the plasma were determined microbiologically.[4]

In another group of animals, surgical transposition of the portal vein and vena cava was performed in order to obtain further information on the causes of the elevated blood ammonia in dogs with a portacaval anastomosis.

METHODS AND RESULTS

Group I: Ammonia Intoxication. *Method.* Under sodium Nembutal anesthesia, portacaval anastomoses were constructed in a group of 5 unselected mongrel dogs. Similar unoperated animals served as controls. Ammonia intoxication was induced by the intravenous infusion of 2 per cent ammonium chloride at a rate of 0.125 mEq. per kilogram of body weight per minute until a convulsion occurred. Immediately thereafter, 20 per cent monosodium glutamate (½ gram per kilogram) was given to two animals, one a

* From the Surgical Research Laboratories of the University of California School of Medicine, San Francisco. This study was supported by the Christine Breon Research Fund.

monium citrate tolerance curves and comparing them with postoperative studies can these sources of error be obviated

Occasionally patients with portacaval shunts given ammonium citrate by mouth may develop clinical signs similar to those found in impending hepatic coma As the blood ammonia reaches abnormally high levels these patients may feel drowsy, develop muscle twitchings, and abnormal electroencephalographic tracings. Such a clinical picture is undoubtedly related to the syndrome of meat intoxication noted in Eck fistula preparations[7] and in cirrhotics after protein or ammonium salt ingestion [3]

Substances other than ammonia might be utilized in a test such as we have described The testing material must be absorbed readily from the intestinal tract, be quantitatively altered by even the most damaged liver cells, and be easily measured. Galactose was considered, but the inability of poorly functioning liver cells to convert this saccharide militated against its use

Other methods which might be utilized for such tests of portal blood diversion are catheterization of the vena cava above and below the shunt with a comparison of concentrations of some material absorbed from the intestinal tract, such as glucose, or a radioactive isotope These methods seem more difficult than the one we have described

SUMMARY

1 A method has been described for determining the patency of portacaval shunts, utilizing the determination of blood ammonia levels following the ingestion of ammonium citrate.

2 This test has proven to correlate with the patency of such vascular shunts both in animals and in man.

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Table 1. Summary of Findings in Dogs Given Ammonium Chloride Infusions as Compared to Those in Normal Animals

ANIMAL	BLOOD AMMONIA LEVELS AT TIME OF CONVULSIONS (mcg./NH_3-N/ml.)	BLOOD pH AT TIME OF CONVULSION	RATE OF RECOVERY (*minutes*)	BLOOD UREA NITROGEN BEFORE INFUSION (mg./100 ml.)	AVERAGE MAXIMUM RISE DURING INFUSION
Normal dog					
Average	22 4 (6*)	7 18	82	20 7 (5*)	6.15
Range	20 2–26 2		57–92	16 2–24.0	
Portacaval anastomosis					
Average	34 6 (8*)	7.18	145	14.8 (5*)	6 9
Range	23.4–52 2		105–180	8.0–20 0	

* Indicates number of determinations

Table 2 Summary of Findings in Dogs with "Meat Intoxication" Compared to Normal Animals

ANIMAL	BLOOD AMMONIA (mcg./NH_3-N/ml.)	PLASMA GLUTAMINE (mg./100 ml.)	PLASMA GLUTAMIC ACID (mg./100 ml.)
Normal			
Average	1.27 (34*)	9 6 (31*)	1.3 (31*)
Range	0 37–2 49	5 9–11 9	0.1–3.7
Portacaval anastomosis (asymptomatic)			
Average	2 90 (12*)	11.4 (8*)	1.4 (11*)
Range	0.49–4.70	4 3–14 2	0.1–2 3
Portacaval anastomosis (symptomatic)			
Average	4.28 (8*)	20.9 (9*)	1.2 (9*)
Range	1.59–8.19	13 3–31.6	0.1–3.6

* Indicates number of determinations.

normal control, the other an animal having a portacaval anastomosis; 60 ml. of a 10 per cent solution of sodium succinate was given to a second pair; and 100 ml. of a 2 per cent solution of ketoglutaric acid to a third. Each of these solutions was administered over a 30 minute period. A fourth pair of dogs received only the ammonium chloride infusions. All of the dogs were observed for changes in their physical status, and blood specimens were taken at the time of convulsion as well as at intervals of 15 to 20 minutes thereafter until the animals were reacting normally. The blood pH, ammonia and urea nitrogen, and the plasma glutamine, glutamic acid and, in some instances, aspartic acid were then measured in each sample of blood.

Results. While receiving the ammonium chloride, the animals passed through a stage of excitement and exhibited the physical signs of increasing acidosis However, they remained responsive until the time of convulsion, when a grand mal type of seizure associated with opisthotonos occurred. There appeared to be no significant difference in the time of onset of convulsion between the normal control dogs and those with a portacaval anastomosis (average time for 5 normal dogs, 35 minutes, 5 dogs with a portacaval anastomosis, 31 minutes). During recovery the animals exhibited extensor rigidity of the extremities and an abnormal gait These symptoms immediately preceded apparent complete recovery, at which time the gait became normal and the animals responded to commands. This progression of the signs of central nervous system disturbances was observed both in the control dogs and in those with a portacaval anastomosis.

The significant laboratory findings are given in Table 1. During the administration of ammonium chloride, the concentration of ammonia in the blood rose steadily to peak values at the time of convulsion, concurrently, the pH of the blood showed a decline which was greatest at the time of convulsion Usually the pH returned to almost normal within 40 to 60 minutes, which was 20 minutes to an hour before the animals had normal physical responses.

It is significant that the time required for complete recovery as well as for the blood ammonia to return to a normal range after intoxication induced by the intravenous administration of ammonium chloride was much longer in the dogs with a portacaval anastomosis than in the normal control animals. However, the rate of recovery of those animals treated with monosodium glutamate, sodium succinate, or ketoglutarate was not significantly different from that of the animals receiving only ammonium chloride.

The blood urea nitrogen was slightly increased following the infusion of ammonium chloride, with the maximum elevation appearing at the time of convulsion or shortly thereafter No significant differences in the plasma glutamine and glutamic acid levels were found in a comparison of the normal animals and those with a portacaval anastomosis prior to the infusion of ammonium chloride. After the intravenous administration of sodium succinate or ketoglutarate, the concentration of glutamine and glutamic acid in the plasma remained unchanged or was only slightly elevated. However, in those animals given monosodium glutamate, the plasma glutamic acid concentrations rose to levels 40 to 50 times those of the baseline values but dropped rapidly and returned to normal in 3 of 4 animals by the time of physical recovery. In these same dogs, there was also an elevation in the glutamine content of the plasma which remained even after apparent recovery. Aspartic acid in the plasma was detected only after the administration

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Range	0 37–2 49	5.9–11.9	0.1–3.7
Portacaval anastomosis (asymptomatic)			
Average	2 90 (12*)	11.4 (8*)	1.4 (11*)
Range	0 49–4 70	4.3–14.2	0.1–2 3
Portacaval anastomosis (symptomatic)			
Average	4.28 (8*)	20 9 (9*)	1.2 (9*)
Range	1.59–8.19	13.3–31.6	0.1–3 6

* Indicates number of determinations.

of monosodium glutamate, when, in both normal animals and those with a portacaval anastomosis, a small but measurable rise in this compound occurred which persisted while glutamic acid remained high

Group II: "Meat Intoxication." *Method* The syndrome of "meat intoxication" was induced by feeding raw horsemeat to 4 dogs with a portacaval anastomosis for a period of 2 to 6 days. Three of these dogs then received monosodium glutamate intravenously at a rate of ½ gram per kilogram of body weight per hour, or sodium succinate as a 10 per cent solution at a rate of 80 ml per hour A control infusion of 200 ml of normal saline was given to one dog with portacaval anastomosis having signs of "meat intoxication." The solutions of monosodium glutamate and sodium succinate were also administered at a similar rate to normal dogs The physical status of the animals was correlated with blood levels of ammonia and urea nitrogen and plasma glutamine, glutamic acid and aspartic acid Blood samples were drawn immediately preceding the infusions, at intervals of 15 to 30 minutes during the infusion, and at hourly intervals thereafter for 3 to 4 hours.

Results Following the induction of "meat intoxication," animals with a portacaval anastomosis showed central nervous system disturbances identical with those which the animals passed through during the recovery phase of ammonium chloride toxicity as described in the results of the group I experiments Recovery took place one to two days after placing the animal on the regular kennel diet.

The levels of ammonia in the blood of animals with portacaval anastomosis exhibiting symptoms of "meat intoxication" were found to be elevated when compared to a similar group of dogs with portacaval anastomosis but without symptoms of "meat intoxication" or to normal dogs (Table 2). Similar differences in the glutamine content of the plasma were also found in these three groups of animals (Table 2)

On seven occasions, infusions of 2 per cent or 4 per cent monosodium glutamate were given at a rate of ½ gram per kilogram of body weight per hour to animals with portacaval anastomoses having symptoms of "meat intoxication" and elevated blood ammonia In six instances, a slight depression of the ammonia values occurred at the onset of infusion, with a maximum depression 60 or 90 minutes later The content of ammonia in the blood then either reached a plateau or tended to rise again toward the basal value. Continuation of the infusion beyond one hour at the same rate did not cause further depression of the blood ammonia levels Glutamic acid values rose five to ten times normal during the infusion, but returned to the basal value within 30 minutes after the infusion was discontinued. Blood glutamine values also became somewhat elevated above the control levels during the infusion of monosodium glutamate and tended to remain slightly higher for 3 to 4 hours

When sodium succinate was administered to 2 animals having central nervous system manifestations of "meat intoxication," it produced a temporary fall in blood ammonia in one of them The plasma glutamic acid levels remained unchanged, but glutamine decreased slightly.

Neither monosodium glutamate nor sodium succinate significantly changed the physical status of the animals with portacaval anastomosis that had signs of "meat intoxication." A control infusion of 200 ml. of physiologic saline into a similar animal produced no significant effect on the ammonia,

glutamine or glutamic acid levels. When monosodium glutamate was given to a dog having a portacaval anastomosis without signs of "meat intoxication" and 2 normal dogs, there was no significant effect on the blood ammonia and only a moderate increase occurred in the plasma glutamine. The increased levels of plasma glutamic acid and glutamine that were found after the infusion of monosodium glutamate into the animals without signs of "meat intoxication" were within the ranges previously reported for those with signs of "meat intoxication."

When sodium succinate was given intravenously to normal animals, some decrease occurred in the plasma glutamine levels, but there was no alteration of plasma glutamic acid or blood ammonia levels.

Group III: Transposition of the Portal Vein and the Vena Cava. *Method.* Three dogs were operated upon under sodium Nembutal anesthesia to produce complete transposition of the portal vein and the inferior vena cava.[1] Systemic blood specimens were drawn for ammonia determinations before the operation and from the portal vein and the vena cava at the time of operation Postoperatively, the animals were fed raw horsemeat for 4 to 6 weeks, and systemic blood ammonia determinations were made immediately after the operations and at approximately 3 week intervals thereafter.

Results When samples were taken at the time of operation for transposition of the portal vein and inferior vena cava, the level of ammonia in the blood obtained from the portal vein was found to be higher than in that obtained from the vena cava or a systemic vein. Following this operation the animals were placed on a diet of raw horsemeat for 4 to 6 weeks. During this period they gained weight and none demonstrated evidence of "meat intoxication" While the animals were on this diet, the blood ammonia levels remained within the normal range.

SUMMARY

1. In control animals and in those with portacaval anastomoses, symptoms closely resembling those seen in so-called "meat intoxication" occurred following the administration of ammonium chloride solution Animals with portacaval anastomoses had a slower recovery period following ammonium chloride–induced convulsions than did normal controls. This was associated with a delayed return of blood ammonia to the basal levels.

2 The intravenous administration of monosodium glutamate, ketoglutarate or sodium succinate did not appear to affect the recovery time or blood ammonia levels following ammonium chloride–induced convulsions.

3 Feeding raw horsemeat for two to six days to animals with portacaval anastomoses produced the syndrome of "meat intoxication" associated with temporary neurologic abnormalities, increased plasma glutamine, and elevation of blood urea nitrogen and ammonia

4 Dogs with a portacaval anastomosis in which "meat intoxication" was induced showed no apparent improvement in their physical status when monosodium glutamate was given intravenously. Slight depression of the blood ammonia was observed in 6 of 7 experiments on dogs receiving monosodium glutamate and in one of 2 dogs receiving sodium succinate.

5 Elevation of plasma glutamic acid occurred during infusion of monosodium glutamate and returned to basal levels 30 minutes after the intravenous infusion was discontinued. Basal plasma glutamine levels were high

in dogs with portacaval anastomosis having "meat intoxication" and although further increases in plasma glutamine while receiving monosodium glutamate were slight, they were maintained as long as 4 hours.

6. Complete transposition of the portal vein and inferior vena cava in 3 dogs produced no significant elevation of fasting blood ammonia levels. It was not possible to induce the syndrome of "meat intoxication" in these animals with prolonged feedings of raw horsemeat.

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STUDIES ON PULMONARY EMBOLISM UTILIZING THE METHOD OF CONTROLLED UNILATERAL PULMONARY ARTERY OCCLUSION*

PAUL NEMIR, JR., H. H. STONE, T. N. MACKRELL, AND H. R. HAWTHORNE

Approximately two and one-half years ago, we initiated studies on pulmonary function using the method of controlled unilateral bronchovascular occlusion. At the meeting of the Clinical Congress in 1953,[1] we presented our experience in the study of 13 patients with particular respect to the main pulmonary artery pressure response to occlusion and to the Valsalva maneuver. Aside from a moderate elevation of the main pulmonary artery pressure following occlusion in certain patients, there were no other untoward responses to total occlusion of the artery to the right or left lung. As the study progressed, it became increasingly apparent that complete occlusion of a

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main branch of the pulmonary artery in the unanesthetized patient with the periarterial nerve fibers intact and with the lung in situ produced in no respect the profound changes which occurred with pulmonary embolism. We felt that we had an ideal method for studying this problem and, accordingly, the study of pulmonary embolism was undertaken as another facet of the project.

MATERIAL

Occlusion of the right or left pulmonary artery has been carried out for varying periods of time on 25 patients up to the present. Of this group, 19 had carcinoma of the lung, 3 had inflammatory pulmonary lesions, 1 had bronchiectasis, one had a large right upper lobe cavity of undetermined etiology and 1 had berylliosis and severe rheumatic heart disease. The youngest patient studied was 37 years of age and the eldest, 72 years of age. The average age was 60 years.

METHOD

The method of unilateral pulmonary artery occlusion for the study of lung function was previously described.[2] For the purpose of studying pulmonary embolism, certain modifications have occurred. Instead of using two catheters, we have recently obtained a triple lumen, size 10½, cardiac catheter with recording lumina both proximal and distal to the occluding balloon This has, of course, greatly facilitated the procedure since, with the use of this triple lumen tube, it is no longer necessary to pass two catheters through the heart. With the use of this catheter we have been enabled not only to record pressure and obtain blood samples for gas analyses both proximal and distal to the inflated balloon, but also to inject substances distal to the inflated balloon

This paper is concerned with the injection of histamine and 5-hydroxytryptamine or serotonin. The method of study was as follows: Following the routine study of pulmonary function,[1,2] arbitrary amounts of the drugs were administered through the distal lumen of the triple lumen catheter. A control study was first performed with the balloon deflated. Histamine (0 55 mg) was injected into the distal lumen and continuous observations

monary artery. Following stabilization of the vital signs, the balloon was then inflated until the right or left pulmonary artery was occluded, and the drug was again injected distal to the occluding balloon. Varying amounts of serotonin were used Our usual policy was to begin with a dosage comparable to the minimum amount previously shown to be effective in cats, i e, 1 microgram per kilogram body weight. We then gradually increased the dosage up to as much as 20 times this amount * Histamine has been injected into five patients and serotonin into three. Every possible precaution, as previously described for the catheterizations, was instituted.

* The serotonin used in these experiments was supplied to us by Dr. J. H. [illegible]

DISCUSSION AND RESULTS

As stated, our previous results indicated that arterial blockade alone in the unanesthetized patient with the lung in situ and the periarterial fibers intact did not produce the signs and symptoms ordinarily associated with pulmonary embolism It seems likely that neurogenic reflexes, set up secondarily in some manner, play a major role in this catastrophe.[3] It is reasonable to assume that some substance is released distal to the clot which is responsible for setting up these reflexes.

Since the lung does not contain histaminase, *histamine* accumulates in this organ and it is actually the chief reservoir in the body for histamine. The use of histamine as a test drug was prompted by the assumption that quantities of this drug might be released from an area of infarction. In all five patients, control injections of histamine without balloon occlusion caused an immediate marked fall in the systemic blood pressure but no alteration of the pulmonary artery pressure. In two of the five patients with control injections of histamine, there was a marked increase in the minute volume of respiration. This latter response must be related to the injection of histamine directly into the pulmonary artery, since it does not occur when histamine is injected into a peripheral vein and further bears out other recent observations that the pulmonary vessels are reactive to certain drugs. With the injection of histamine distal to the occluding balloon in a main branch of the pulmonary artery, there was a variable delayed slight transient fall [illegible] change of the pulmonary [illegible] the balloon, the systemic [illegible] he histamine was trapped distal to [illegible] *in the v*[illegible] untowar[illegible] pulmona[illegible] we felt justified in concluding that the release of histamine distal to a clot was probably not a prominent factor in pulmonary embolism.

A second substance which seemed worthwhile investigating with relation to pulmonary embolism was serotonin, or 5-hydroxytryptamine, a vasoconstrictor substance present in the active state in serum. It is felt by a number of investigators[3] that 5-hydroxytryptamine, liberated locally from disintegrated platelets during the process of blood coagulation, might initiate profound systemic reflex changes in addition to the localized vasoconstriction Blood coagulates whenever a vessel is torn. 5-Hydroxytryptamine, liberated locally from disintegrated platelets, may, by its direct constrictor action on vascular smooth muscle, pull the walls of the vessel together and prevent further hemorrhage However, whenever blood clots within an intact vessel, the vasoconstrictor properties of locally liberated 5-hydroxytryptamine may cause spasm and further reduce blood flow Thus, when a peripheral artery becomes plugged by an embolus and additional clot forms on either side, 5-hydroxytryptamine so formed may be responsible for the undesirable peripheral vasospasm. When blood clots in a pulmonary, cerebral, or coronary vessel, either primarily or about an embolus, the liberation of 5-hydroxytryptamine may produce additional, and possibly a dangerous degree of, vascular obstruction. It would be most apt to produce profound

reflex changes when the blood clots in vessels in intimate association with chemoreceptors sensitive to 5-hydroxytryptamine.

Comroe and his associates[4] have reported that rapid intravenous injection into cats of 5-hydroxytryptamine resulted in one or all of the following effects: bradycardia, hypotension, apnea, bronchial obstruction, and increase in the right ventricular pressure The dosage level has, of course, been somewhat arbitrary. Comroe and his associates found that the minimal amount which was usually effective in cats was 1 microgram of the 5-hydroxytryptamine base per kilogram body weight. Our policy in patients was



Fig 1 Femoral and pulmonary artery response to the injection of histamine distal to an occluding balloon in the left pulmonary artery The top tracing represents the pressure recorded through the proximal lumen of the catheter, i e , the main pulmonary artery pressure The middle tracing represents the pressure recorded through the distal lumen Note in the first block that upon inflation of the balloon the distal pressure drops off to the range of the average pulmonary capillary mean pressure, and a pulmonary capillary or wedge type tracing is obtained (second block). Whereas the fall in systemic blood pressure occurred within 30 seconds following the control [illegible] and there was an immediate precipitous fall in the systemic blood pressure (block four) Note in this block, also, the return of the pressure recorded through the distal lumen toward that recorded through the proximal lumen upon deflation of the balloon Note also that no appreciable pressure changes occurred at any time in the main pulmonary artery.

to begin with an equivalent amount, i e., 70 micrograms for a 70 kilogram patient and to gradually increase the amount injected.

Except for a mild systemic hypertension in the control study, no other effects were noted. There was no alteration of the pulmonary artery pressure Injection distal to the occluding balloon produced no appreciable changes in the systemic or pulmonary circulations or in the respiration, even when used in amounts up to 20 times greater than the minimal effective dose in cats. In one of the three patients there was a very transient tachycardia up to 120 per minute but this lasted only for several minutes. In one patient a very transient increase in the respirations also occurred. Although the number of patients studied has been small, the results were nonetheless unequivocal, and it seems likely that the discrepancy in results between

man and cat is due to factors related to species difference Further studies are in progress with respect to this and other drugs.

SUMMARY AND CONCLUSIONS

1. Complete occlusion of the right or left pulmonary artery in the intact, unanesthetized patient by the use of a balloon-tipped cardiac catheter in no way initiates the profound changes which are seen in severe pulmonary embolism.

2. It is probable that the profound reflex changes which do occur are brought about by the release of a substance distal to the clot.

3. Histamine, which might conceivably be released under such circumstances, is apparently not the substance released, since its injection distal to the occluding balloon was not associated with the profound reflex changes ordinarily seen in pulmonary embolism.

4. 5-Hydroxytryptamine, or serotonin, while producing profound reflex changes in cats consistent with those ordinarily seen in pulmonary embolism, is without appreciable effect in man, at least with the dosages and methods used by us. The discrepancy is apparently related to species difference.

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RELATIONSHIP BETWEEN PULMONARY EMBOLISM AND PULMONARY INFARCTION*

H. DAVID ROACH AND HAROLD LAUFMAN

We have reopened the problem of pulmonary infarction to study two phases of the problem: first, the nature of the interrelationship between the vascular impairment and the underlying condition of the lung, and second, the pathogenesis of pulmonary infarction in the absence of pulmonary embolism

A total of 95 adult mongrel dogs, weighing from 8 to 16 kilograms, were used in our experiments. In order to simulate, as closely as possible, the clinical conditions of pulmonary embolism, large, firm thrombi were made in the animal's own blood stream, and released through the circulation to the pulmonary vessels.

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Our technique for producing thrombi[1] was as follows. Both external jugular veins were exposed for a distance of 8 to 10 cm. in an animal anesthetized with intravenous Nembutal. A rubber-shod bulldog clamp was applied to the proximal end of the exposed segment. If the vein did not distend rapidly, it was gently milked toward the clamp. A total of 0.5 cc. (500 units) of bovine thrombin* was injected through a 27 gauge needle, using three separate sites along the vein. Bleeding from the puncture sites was prevented by slowly injecting while the needle was withdrawn. In addition, the vein segment was massaged gently 4 or 5 times to insure mixing of the thrombin and blood. A palpable clot immediately formed. In our previous work, we found that a delay of 45 minutes after the initial clot formation was necessary before releasing the thrombi into the circulation, in order to produce a large, non-fatal pulmonary embolus. We had found that this time interval was needed for the total inactivation of the excess injected thrombin. If such thrombin-induced clots were released before a lapse of 45 minutes, a soft clot was formed in the right side of the heart, resulting in death.[1]

Care had to be exercised not to fragment the clot by vigorous crushing with the fingers. If the clot was broken up into many small clots before release, death of the animal ensued within 10 minutes, the result of propagating soft clot formation.

The presence and extent of the pulmonary embolus and associated pathology was determined by sacrifice of each animal and by dissection of the pulmonary vascular tree. The lungs were gently filled with 4 per cent Formalin solution and stored in a vat of Formalin for 24 hours. The lungs were sectioned grossly into slices 1 cm. thick and specimens were taken from representative areas. Microscopic sections of 6 micron thickness were cut and stained with hematoxylin and eosin after mounting. Gross and microscopic photographs were taken of each specimen.

CONTROL SERIES: PULMONARY EMBOLISM IN HEALTHY DOGS

Pulmonary emboli were produced in 22 apparently healthy dogs by the method described. The dogs were sacrificed at various intervals after embolization. One dog was sacrificed at 24 hours, 12 at 48 hours, 2 at 72 hours, 4 at 7 days, one at 9 days, one at 14 days and one at 27 days. All animals appeared healthy until sacrificed.

Gross pulmonary emboli were found in each instance. In 17 dogs no gross or microscopic evidence of infarction was found, but five animals had areas of hemorrhage, or pre-infarction.

A small area of atelectasis or pneumonia was found in each of the remaining 5 dogs Two of these animals had been sacrificed at 48 hours, one at 72 hours, one at 7 days, and one at 14 days Within each of these areas, *evidence* of hemorrhagic infarction could be found, although in only 2 was infarction diagnosed from the gross specimen. In addition, one dog had an area of pre-infarction. In the other 3 instances, microscopic section of the atelectatic area revealed infarction, as evidenced by the shadows of necrotic alveolar walls in the atelectatic tissue, and hemorrhage with *some* polymorphonuclear infiltration As early as 48 hours after embolization, a layer of endothelial cells was found to cover the emboli (Fig. 1*B*). Specimens from the

* Parke-Davis Bovine Thrombin, Topical, 1000 units per cc.

animals sacrificed at 7 and 14 days showed beginning recanalization of the emboli

Comment. Pre-infarction or infarction only occurred in areas where a pre-existing patch of atelectasis or pneumonia had been present. Emboli in other portions of the lungs caused no ill effects It has been asserted that arterial circulation and ventilation are parallel functions,[2] i.e., when one is

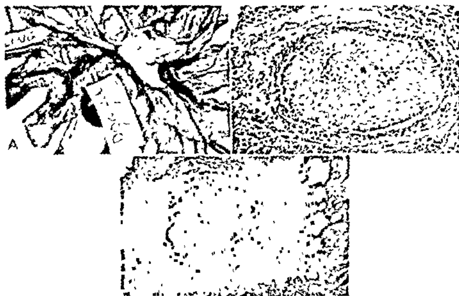


Fig 1 Control pulmonary embolism A, Fresh gross specimen after dissection of pulmonary arterial tree, showing distribution and size of emboli, dog sacrificed at 48 hours B, Photomicrograph (×100) from same specimen, partially attached embolus is seen surrounded by layer of endothelial cells and invaded by fibroblasts C, Photomicrograph (×60) of embolus from dog sacrificed at 7 days, showing almost complete organization of embolus and early recanalization of lumen of artery



Fig 2 A, Fresh gross specimen showing infarction following pulmonary embolism in area of atelectasis, sacrificed at 14 days B, Photomicrograph (×100) from same specimen showing organization of plug in bronchus in area of atelectasis.

decreased, the other is also impaired It has been known for some time that pulmonary arterial circulation is decreased in an area of atelectasis. Collateral circulation by way of the bronchial arteries may aid in maintaining viability under these circumstances, but several investigators[3,5] have reported that the bronchial artery does not dilate after obstruction of a bronchus to a lobe. Hence, an atelectatic area, by virtue of its diminished

aeration and attenuated blood supply, would appear to provide fertile soil for infarction when vascular impairment is superimposed. We believe our control series substantiates this concept.

PULMONARY EMBOLI IN DOGS WITH PNEUMONIA

Twenty-two dogs with distemper were used in this experiment, on the premise that such dogs usually had pneumonia. To test this premise, 8 dogs with distemper were sacrificed and their lungs were examined grossly and microscopically. Pneumonia of varying degrees was found in 5, while in 3 the lungs showed no gross or microscopic evidence of pneumonia.

Pulmonary emboli were produced in 13 dogs with distemper, using the technique described above. Two animals died in 48 hours, seven were sacrificed at 48 hours, one at 4 days, one at 9 days, one at 10 days and one at 11 days.

Seven of the 13 embolized distemper dogs had frank hemorrhagic infarction in pneumonic lobes at postmortem examination. The extent of pneu-



Fig. 2 [illegible] B, Photomicrograph (×60) of infarct of same specimen showing hemorrhage and necrosis of alveoli, many leukocytes present.

monia varied considerably among the animals, ranging from a small pneumonic patch in one lobe to involvement of all seven pulmonary lobes by various phases of pneumonia The presence of infarction could not always be ascertained by gross examination of the fresh specimen, because of the varied appearance of the different stages of pneumonia and hemorrhage. However, on microscopic section, infarction was confirmed in each of these specimens

Of the 6 distemper animals which did not develop infarction following embolism (consisting of 4 sacrificed at 48 hours, one at 10 days and one at 11 days), three were found free of gross or microscopic evidence of pneumonia The other 3 dogs in this category had varying stages of patchy bronchopneumonia, with some hemorrhage into pneumonic areas [illegible]

[illegible] embolized lung with pneumonia are decreased ventilation, diminished arterial circulation, edema and infection, it becomes necessary to evaluate each of these factors from the standpoint of quantitative importance. It is further logical to determine whether under properly balanced

circumstances these factors alone might cause actual infarction without the necessity of a superimposed embolus.

A series of experiments was undertaken to examine each of these factors They will only be outlined here, since they will appear in detail in a future communication.[6]

Decreased aeration of one lobe was produced in 8 dogs by ligating the right lower lobe bronchus distal to the first bronchial branch. *Five of these dogs were subjected to pulmonary embolization. In no instance did actual* infarction occur. Our results indicated that a limited decrease in ventilation alone does not materially influence the formation of infarction following embolization.

Decreased aeration of a lobe and ligation of a branch of the corresponding pulmonary artery in 3 animals resulted in infarction in each instance Ligation of the artery without decreased ventilation does not cause infarction.[3] The occurrence of infarction when decreased blood supply is superimposed

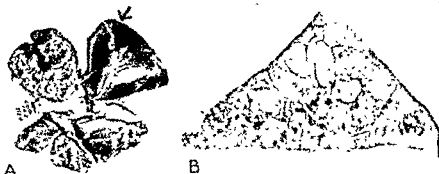


Fig 4 *A*, Gross appearance of fresh specimen of lung 7 days after ligation of pulmonary venous branches of right lower lobe and unsterile plug of corresponding bronchus Infarct indicated by arrow No embolism was employed. *B*, Unmagnified photograph of section through infarct in same specimen

upon decreased aeration is apparently dependent upon the degree of completeness *of either or both these factors*

Unsterile plugs were placed in the right main bronchus and pulmonary embolism was superimposed, to test the effect of embolization in the presence of decreased aeration and infection This was carried out in 14 dogs, while 5 dogs with plugs in the bronchus and no embolus served as controls The nature and incidence of infarcts and pre-infarcts in this series differed little from that of our distemper series, emphasizing, we believe, the importance of infection and decreased aeration in the production of infarction following embolization

Ligation of both anterior branches of the pulmonary vein of the right lower lobe plus placement of an unsterile cotton plug in the right main bronchus resulted in infarction in 5 of 6 dogs and parenchymal hemorrhage in the remaining dog. Ligation of the vessels without unsterile occlusion of the bronchus resulted in hemorrhagic infiltration without infarction. It appeared from this experiment that if infection, decreased aeration and congestion are severe enough, pulmonary infarction may result in the absence of pulmonary embolism.

SUMMARY

Our experiments suggest that a firm, bland pulmonary embolism is more likely to lead to pulmonary infarction when infection, decreased aeration or congestion are present in the lung. When pneumonia exists prior to embolization, infarction usually results. Decreased aeration, infection and congestion may be severe enough to result in infarction in the absence of pulmonary embolism.

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THE RENAL HEMODYNAMIC RESPONSE TO HYPOTHERMIA AND TO CLAMPING OF THE THORACIC AORTA WITH AND WITHOUT HYPOTHERMIA*

GEORGE C. MORRIS, JR., JOHN H. MOYER, DENTON A. COOLEY, AND H. LE ROY BROCKMAN**

The increasing clinical application of hypothermia has stimulated study of its physiologic effect. Total oxygen consumption diminishes proportionately with fall in body temperature provided shivering is inhibited.[1] Cardiac output decreases as the temperature is reduced.[2] In the current study renal blood flow, glomerular filtration rate and urine volume were measured in dogs before, during and after artificially induced low temperature states. In a second group of animals renal function was determined before, during and after clamping of the thoracic aorta with and without cooling. Mean blood pressure alterations are correlated with the renal clearances.

METHODS

Female dogs were hydrated with water (40 cc./kg) introduced into the

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stomach with a Levin tube. After one hour the dogs were anesthetized with intravenously administered pentobarbital (30 mg./kg.). Hypothermia was effected with refrigeration blankets Additional injections of pentobarbital were used when needed to counteract shivering A continuous automatic rectal temperature graph was recorded. Respiration was maintained by means of a pump delivering intermittent positive pressure through an endotracheal tube. Blood pressure was measured by direct femoral arterial manometry (mercury). Renal blood flow was derived from renal plasma flow measured by para-aminohippurate (PAH) clearance Glomerular filtration rate was determined by creatinine clearance Methods and techniques have been previously described [3]

Control records are averages of three consecutive ten minute collection periods Two consecutive ten minute periods were obtained when the temperature was stabilized at 26° to 28° C. in thirteen dogs In five of these dogs two more ten minute periods were obtained during the last twenty minutes of two hours of hypothermia. Two periods were obtained immediately after rewarming in six dogs and after twenty-four hours in four dogs. Similar observations were obtained in the ten normal temperature dogs submitted to one hour of thoracic aortic clamping The collection periods consisted of control, during clamping, the first and second twenty minutes after clamp release and two, four, and twenty-four hours after release. Aortic clamping was carried out aseptically with a vascular clamp through an appropriate left intercostal space incision The clamp was placed across the aorta above the diaphragm In another group of five dogs with combined hypothermia and clamping, control periods were followed by one hour of hypothermia at 26° to 28° C. Collecting periods were obtained during hypothermia, during one hour of thoracic aortic occlusion, immediately after rewarming, and two, four, and twenty-four hours after rewarming.

RESULTS

Hypothermia. The mortality rate in the group of dogs with controlled hypothermia at temperatures of 26° to 28° C for two hours was low. Several dogs developed ventricular fibrillation and were discarded from the study The results are recorded in Table 1 All thirteen animals developed at least a moderate hypotension The average mean blood pressure for the group during hypothermia was 68 per cent (expressed as average mean percentage of control). Renal blood flow fell to 31 per cent of control during the first hour of hypothermia and remained depressed during the second hour. Immediately after rewarming, mean blood pressure rose to 93 per cent and renal blood flow to 67 per cent In the four animals followed twenty-four hours, renal blood flow returned to normal Glomerular filtration rate paralleled renal blood flow with a severe depression during hypothermia and returned to normal after twenty-four hours. Water excretion, however, continued unabated during hypothermia reflecting diminished tubular reabsorption.

Thoracic Aortic Occlusion without Hypothermia. The results are tabulated on Table 2. During the hour of thoracic aortic clamping below the subclavian artery, femoral mean blood pressure fell to a range of 14 to 28 mm. Hg. Renal function regularly became nil during the clamping period. The mortality rate during the first twenty-four hours after release of the clamp was exceedingly high and only those dogs demonstrating reasonable

Table 1. Effects of Two Hours of Hypothermia (26° to 28° C.)

	Mean Blood Pressure mm Hg C	H_1	H_2	T_1	R_6	Glomerular Filtration Rate ml./min C	H_1	H_2	T_1	R_6
1	132	100				13	19			
2	124	91				39	19			
3	137	80				47	20			
4	132	88				47	25			
5	135	87				18	18			
6	139	78	84	—	—	63	21	19	—	—
7	145	100	—	—	—	34	11	—	—	—
8	81	70	—	70	—	25	11	—	17	—
9	110	88	84	98	—	13	1	20	33	—
10	157	85	100	150	117	12	14	18	17	51
11	125	110	117	113	95	56	25	26	37	65
12	131	60	65	140	117	46	8	13	34	65
13	141	92	—	124	98	38	16	—	25	41
Mean	130	87	90	116	107	44	16	19	27	56
Mean % of control		68	69	93	78		37	39	65	122

	Renal Blood Flow ml /min C	H_1	H_2	T_1	R_6	Urine Volume cc /min C	H_1	H_2	T_1	R_6
1	372	161				0 8	0 8			
2	203	63				1 9	1.3			
3	302	95				1 9	1 5			
4	194	41				3 3	2 3			
5	325	90				1 7	1.1			
6	100	86	78	—	—	1 3	1.5	1 4		
7	190	58	—			0 8	1 4	—		
8	132	64	—	117	—	0 3	0 3	—	0 4	
9	208	—	89	155	—	0.4	0 1	0 8	1.0	—
10	226	80	91	94	305	0 5	0.6	0 6	0 6	0 5
11	313	93	90	220	335	1 1	2 0	2.4	2.9	1.2
12	379	65	102	255	312	0 7	0 3	0 4	1.4	1.2
13	239	95	—	141	290	0 6	0.4	—	1.6	0 7
Mean	268	83	90	164	311	1.2	1.0	1.1	1.3	0 9
Mean % of control		31	32	67	111		93	111	206	124

C = Control; H_1 = first twenty minutes at temperature 26°-28° C.; H_2 = last twenty minutes of two hours of hypothermia at temperature 26°-28°C.; T_1 = *immediately after rewarming*; R_6 = after twenty-four hours.

Table 2. Effects of Occlusion of Thoracic Aorta with and without Hypothermia

	Mean Blood Pressure mm Hg							Glomerular Filtration Rate ml/min							Renal Blood Flow ml/min							Urine Volume cc/min						
	C	S	R_1	R_2	R_3	R_4	R_5	C	S	R_1	R_2	R_3	R_4	R_5	C	S	R_1	R_2	R_3	R_4	R_5	C	S	R_1	R_2	R_3	R_4	R_5
ONE HOUR DISTAL THORACIC OCCLUSION WITH NORMAL BODY TEMPERATURE																												
1	135	20	145	150	—	—	108	48	1	6	19	—	—	54	260	4	16	105	—	—	232	3.1	0.1	1.0	1.8	—	—	2.7
2	120	28	100	90	120	—	—	30	*	16	21	22	—	—	295	*	90	89	152	—	—	0.8	0	1.0	0.8	1.0	—	—
3	118	14	120	130	140	130	—	43	*	20	21	30	31	—	258	*	125	127	139	177	—	0.4	0	0.4	0.5	1.4	1.5	—
4	120	20	85	88	100	87	—	65	*	10	43	37	36	—	313	*	216	179	127	165	—	1.1	0	1.3	1.3	1.4	1.8	—
5	154	22	78	100	108	162	131	49	*	35	46	39	39	44	260	*	215	250	144	169	203	0.4	0	1.8	1.4	0.8	0.8	0.8
6	150	20	50	80	90	—	—	53	1	2	12	25	—	—	308	*	15	89	139	160	—	1.0	0.2	0.2	0.4	1.0	—	—
7	133	26	110	124	118	161	95	56	*	*	20	26	36	66	260	*	*	169	163	250	339	0.5	0	0.1	0.5	0.4	0.4	1.1
8	160	24	116	122	126	—	131	29	*	4	11	10	—	21	178	*	*	59	120	—	111	0.6	0	0	0.5	0.2	—	0.5
9	120	20	70	110	100	—	100	68	5	29	25	—	—	73	314	38	183	110	—	—	297	2.1	0.3	0.9	0.9	—	—	1.1
10	129	26	46	156	156	—	112	67	*	*	20	22	—	39	392	*	*	116	114	—	226	1.8	0	0	0.4	0.4	—	0.7
Mean	134	22	92	115	118	135	113	51	0	15	24	26	36	50	284	4	86	129	137	184	238	1.2	0	0.7	0.9	0.8	1.1	1.2
Mean % of control		17	70	87	89	102	81		4	31	48	55	68	93	—	1	30	46	51	67	87	—	4	91	104	130	205	114
ONE HOUR DISTAL THORACIC OCCLUSION DURING TWO HOURS OF HYPOTHERMIA (26°-28° C.)																												
1	132	100	29	100	90	126	95	43	19	2	8	18	34	69	372	161	13	46	126	189	470	0.8	0.8	*	1.0	1.8	1.4	1.9
2	124	91	28	53	80	118	—	39	19	*	*	2	15	—	203	63	*	*	*	57	—	1.9	1.3	*	*	0.1	0.6	—
3	137	80	15	75	80	120	—	47	20	*	12	17	31	—	302	95	*	54	97	134	—	1.9	1.5	*	0.3	0.6	1.4	—
4	132	88	15	93	—	145	103	47	25	*	2	—	15	47	194	44	*	*	—	57	316	3.3	2.3	*	0.1	—	0.6	1.1
5	135	87	23	75	82	111	—	48	18	*	6	13	22	—	325	90	*	64	69	178	—	1.7	1.1	0.1	0.6	0.7	1.0	—
Mean	132	89	22	79	83	130	99	45	20	0	6	13	23	58	279	90	13	55	97	123	393	1.9	1.4	0.1	0.5	0.8	1.0	1.5
Mean % of control		68	17	60	63	99	75	—	45	0	12	28	52	130	—	31	0	17	29	44	145	—	76	6	45	76	72	136

C = Control; S = during aortic occlusion; H_1 = during hypothermia (26°-28°C.); R_1 = first 20 minutes after release of clamp; R_2 = second 20 minutes after release of clamp, R_3 = two hours after release of clamp (rewarmed in the hypothermic group); R_4 = four hours after release of clamp; R_5 = 24 hours after release of clamp. * = Excretion too small to measure

recovery are included in this report. Ultimate survival usually could be clinically predicted by the severity of watery, bloody diarrhea developing immediately after release of the clamp. Renal function returned to normal in dogs which were in good condition twenty-four hours after sixty minutes of aortic occlusion.

Thoracic Aortic Occlusion with Hypothermia. The results are tabulated in Table 2 (hypothermic portions of experiments also listed in Table 1). The mortality rate was similar to that observed following clamping of the aorta without hypothermia. Mean femoral arterial blood pressure ranged from 15 to 29 mm. Hg during thoracic aortic occlusion. Renal function following hypothermia and aortic occlusion remained greatly depressed for two hours, but returned to normal in the two dogs studied at twenty-four hours.

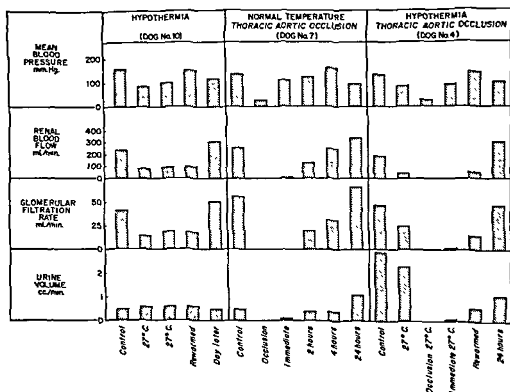


Fig 1. Effects of hypothermia and of occlusion of thoracic aorta, with and without hypothermia, on renal hemodynamics of dogs.

SUMMARY AND CONCLUSIONS

Hypothermia in dogs at a temperature level of 26° to 28° C. promotes moderate hypotension. A severe depression in renal blood flow and glomerular filtration rate takes place during hypothermia. Renal function does not completely recover during the first few hours after rewarming but reaches normal values within twenty-four hours. Urine flow is not depressed in the cooled state and reflects diminished tubular reabsorption. Renal function recovers within twenty-four hours following one hour of distal thoracic aortic occlusion. Hyperthermia does not alter renal recovery following thoracic aortic occlusion.

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THE USE OF HYPOTHERMIA IN THE PREVENTION OF BRAIN DAMAGE FOLLOWING TEMPORARY ARREST OF CEREBRAL CIRCULATION: EXPERIMENTAL OBSERVATIONS*

ROBERT G. PONTIUS, ROBERT D. BLOODWELL, DENTON A. COOLEY, AND MICHAEL E. DE BAKEY**

Recent developments in arterial surgery directed toward excision of diseased segments and their replacement with homografts have provided a much more favorable outlook for these conditions There are, however, certain limitations to the application of this form of therapy, one of the most important of which arises from the technical necessity of arresting circulation during performance of the procedure. As a consequence of this temporary interruption of blood flow, serious ischemic damage to the tissues distal to the point of occlusion may take place depending upon a number of factors, including particularly the period of occlusion and the sensitivity of the tissues to anoxia The tissues of certain vital organs, for example, such as those of the central nervous system, are highly vulnerable to damage from temporary ischemia and even relatively short periods of interruption of blood flow to these tissues may produce grave neurologic disturbances. This problem thus assumes particular importance in the application of excisional therapy for lesions involving arteries such as those about the aortic arch that supply such vital organs

One approach to this problem that seems promising is through the use of hypothermia and the consequent reduction of oxygen demand by the tissues of the central nervous system.[2] Previous studies have shown that hypothermia has a definite protective influence against ischemic damage of the spinal cord following high aortic occlusion.[1, 3] Accordingly these studies were undertaken to determine the effectiveness of hypothermia in preventing cerebral damage following temporary arrest of the cerebral circulation in the dog.

Extirpation of the common or internal carotid artery in man may be expected to result in a mortality of 40 to 50 per cent.[7] In lower animals,

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however, such a procedure is followed by minimal disturbance, owing to the presence of extensive collateral circulation through the vertebral and accessory pathways.[5] For these reasons much of our experimental work was directed toward the development of a suitable preparation which would produce neurologic sequelae in a substantial number of cases as controls.

METHOD

Mongrel dogs ranging from 7 to 14 kilograms in weight were used in all the experiments. Anesthesia was obtained by intravenous Nembutal, 30 mg. per kilogram of body weight, and artificial respiration was provided by

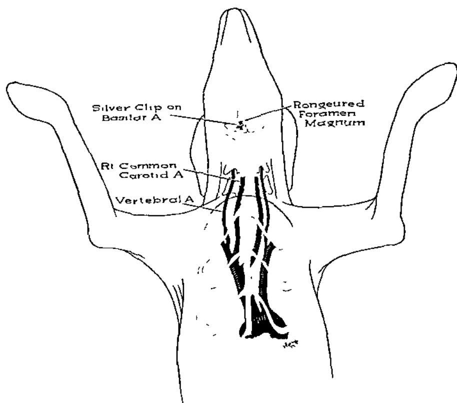


Fig. 1 Diagrammatic representation of points of occlusion.

means of endotracheal tube and mechanical insufflator. Preliminary observations in 30 dogs revealed no detectable neurologic changes following periods of occlusion up to one hour of the brachiocephalic, subclavian, carotid and vertebral arteries in various combinations. It thus became apparent that further impediment to cerebral blood flow was necessary. Accordingly the following experiments were performed:

Group I. A permanent silver clip was placed upon the basilar artery and a tourniquet applied around the neck. The basilar artery was approached anteriorly through the foramen magnum with the structures of the neck retracted laterally, and the anterior portion of the occipital bone was carefully rongeured away for exposure. The dura was transversed to apply the clip The tourniquet about the neck was occluded for 30 minutes at a pressure of 900 mm Hg.

Group II. In addition to the procedure employed in group I, the carotid and vertebral arteries were temporarily occluded at the root of the neck for

a similar period of time to reduce deep circulation not affected by the tourniquet (Fig. 1).

Group III The procedure used on these animals was the same as that done for those in group II but in addition a state of hypothermia was induced with rectal temperature ranging from 25° to 31° C. during the period of occlusion. Hypothermia was produced by wrapping the animals in a rubberized blanket through which a refrigerant solution was circulated. Following completion of the experiment rewarming was accomplished with the same blanket through which hot water was circulated.

RESULTS

In group I five of the six dogs made spontaneous recovery with no apparent neurologic disturbances (Table 1). The sixth animal, however, developed a dilated pupil during occlusion and, although recovering from anesthesia, died with generalized convulsions which were set off by auditory or tactile stimuli.

Table 1. Results Following Temporary Occlusion of Cerebral Circulation

EXPERIMENTS	NUMBER OF ANIMALS	RECOVERED	DIED	
			NUMBER	PER CENT
Group I	6	5	1	16.7
Group II	9	3	6	66 6
Group III	9	9	0	0

In group II three of the nine dogs made eventual complete recovery (Table 1). Of these, two resumed respirations promptly while the third required artificial respiration for 8 hours. All of the fatal group developed a dilated pupil within 3 to 9 minutes after occlusion. Three of these failed to resume respiration in from 2 to 6 hours. Although respiration returned in two of the others, they subsequently developed convulsions and died in 24 hours. The remaining animal died in 6 hours without convulsions.

In group III all of the nine animals treated with hypothermia recovered and none developed a dilated pupil (Table 1). All were observed for a period of a month with no obvious neurologic changes.

[illegible] group II and group III would appear to be due to the use of hypothermia in the latter as this was the only difference in these experiments.

DISCUSSION

Previous investigations along these lines have indicated the usefulness of hypothermia in preventing ischemic damage to the spinal cord following temporary arrest of the circulation in the aorta.[1, 9] Thus the incidence of paraplegia and death following high aortic occlusion, just distal to the left subclavian, for one hour was reduced from 76 per cent in a group of 50 [illegible] to 25 per cent in a group of 47 hypothermic animals.[9] Even [illegible]

cally there is also reason to believe that hypothermia may have protective value in preventing ischemic cord damage following temporary cross-clamping of the thoracic aorta. The threat of this complication has been demonstrated following such a procedure in the treatment of extensive aneurysms of the thoracic aorta.[3, 6] In an effort to reduce this hazard hypothermia has been employed in 6 cases of aneurysms of the thoracic aorta arising in the distal part of the arch in which resection with homograft replacement was done.[4] In spite of the fact that the aorta was occluded at the level of the left subclavian artery or just distal to the left common carotid artery for periods up to one hour, none developed any evidence of cord damage.

As has been indicated above, our preliminary studies showed that in the dog occlusion of the major branches of the aortic arch is well tolerated, owing presumably to the presence of extensive collateral circulation. Even with the additional occlusion of the basilar artery only one of six animals

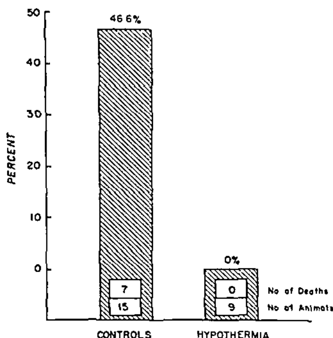


FIG. 2. Mortality in animals following temporary occlusion of cerebral circulation.

developed ischemic cerebral changes. Indeed, only after the occlusion of additional arterial pathways as was done in the group II experiments was it possible to reduce cerebral circulation sufficiently to produce an appreciable degree of ischemic damage. This was usually manifested by dilatation of the pupil in 3 to 9 minutes. Following restoration of cerebral blood flow the pupil may gradually decrease in size, but spontaneous respiration may not take place and death occurs upon termination of artificial respiration. In others while respiration may be resumed, the animal develops a state of tension responding to auditory and tactile stimuli with bursts of tonic convulsions and dying in a state of exhaustion within 6 to 24 hours.

Since none of these manifestations occurred in the comparable group of hypothermic animals it would appear that this procedure has a significant protective value in preventing ischemic damage to the brain in dogs following temporary arrest of cerebral circulation (Fig. 2). These observations conform with those recently reported by Parkins, Jensen and Vars,[8] who

found that brain cooling to 20° C. provides adequate protection against 30 minutes of complete circulatory occlusion. It would appear, therefore, that in the dog hypothermia has protective value in preventing ischemic brain damage following temporary arrest of the circulation. Accordingly it deserves further study as a promising means of overcoming this hazard in the clinical attack upon vascular lesions involving the major arterial channels to the brain.

SUMMARY

1. Studies were undertaken to determine the effectiveness of hypothermia in preventing brain damage following temporary arrest of the cerebral circulation. Following preliminary observations which revealed that in the dog temporary occlusion of the brachiocephalic, subclavian, carotid and vertebral arteries up to one hour produced no neurologic disturbance owing presumably to extensive collateral circulation, three groups of experiments were performed. In the first group the basilar artery was occluded and a tourniquet applied around the neck at a pressure of 900 mm. Hg for 30 minutes. In addition to this procedure in the second group, temporary occlusion of the carotid and vertebral arteries was done. The third group was similar to the second group except that the body temperature of these animals was reduced to about 25° to 31° C.

2. There was a striking increase in mortality from 16.7 per cent in the first group of animals to 66.6 per cent in the second group, indicating that the procedure in the latter group was much more effective in obstructing cerebral circulation. In the third group of animals in which the same experiment was done as in the second group except that hypothermia was used, there were no deaths and no other evidence of brain damage.

3. On the basis of these experiments it is concluded hypothermia appears to have protective value in the dog against ischemic damage to the brain following temporary arrest of cerebral circulation.

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VASCULAR GRAFTS

THE USE OF HETEROGENOUS VEIN AND ARTERY GRAFTS SUPPORTED BY A PLASTIC SPONGE*

JOHN H. MORTON AND EARLE B. MAHONEY

The increasing interest in surgical resection of portions of the arterial tree has led in recent years to a renewed search for satisfactory substitutes to replace the removed vessels. In work with smaller vessels many surgeons prefer an autogenous arterial or vein graft, but no autogenous vessel of satisfactory caliber is available when a portion of the aorta must be replaced. At present the most widely used substitute for aortic grafting is a homologous segment of aorta. A satisfactory homograft should have undergone no degenerative changes, and the donor's death should not be due to an infectious or neoplastic process. Most acceptable human vessels, therefore, come from young people who meet with accidental death, and the supply of such vessels is necessarily limited.

To overcome this difficulty some interest has developed in the use of other materials. Voorhees, Jaretzki and Blakemore[4] have employed tubes made of Vinyon "N" cloth to replace segments of the dog's aorta successfully, and Shumacker and King[3] have used plastic tubes made from nylon and polythene in experimental animals and human patients with good results.

At the 1953 Surgical Forum, Hufnagel, Rabil and Reed[2] reported the use of arterial heterografts in animal and human studies. They successfully transplanted calf, pig, lamb and human arterial segments into the dog's abdominal aorta. Creech et al.[1] recently reported a study of heterografting in which most of their experiments involved transplantation of arteries from pig to dog. All 13 common carotid artery grafts became occluded as did 3 of 21 in the abdominal aorta. Of 18 patent aortic grafts 9 were dilated when examined at different intervals in the postoperative period.

The present study was undertaken to provide further data on heterografting using both arterial and venous segments in the abdominal aorta. In an attempt to prevent aneurysmal dilatation of the grafts, they were wrapped in an inert plastic sponge of polyvinyl formal† before closing the abdomen

MATERIALS AND METHODS

In one experiment a segment of human saphenous vein, removed during a vein stripping procedure, was used as the graft. All other grafts were obtained from young pigs which had been previously subjected to surface flash burns in another study. The grafts were obtained under aseptic conditions when the pigs were sacrificed. Segments of the aorta and inferior vena cava were placed in Ringer's solution to which 1,000,000 units of aqueous penicillin had been added, and they were then stored in an ice box at 3° C.

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† Polyvinyl formal sponge supplied by O-Cel-O Division of General Mills, Inc., Buffalo, New York.

until used. The period of storage varied from a few hours to 13 days, the majority of grafts being used between 1 and 8 days after they were obtained.

Forty-three adult mongrel dogs of both sexes, varying between 7 and 24 kilograms in weight, were used as subjects. In one instance an aortic graft was placed in the thoracic aorta under hypothermia. In all other experiments the abdominal aorta below the renal arteries was used. Operations were performed using intravenous sodium pentobarbital anesthesia under aseptic conditions The abdominal aorta was exposed through a midline or left rectus muscle splitting incision, and a suitable segment of aorta freed from the retroperitoneal structures. When this had been done, the aorta was clamped above and below with Potts aortic clamps and divided between the clamps. A short segment of the aorta was removed and a somewhat longer graft substituted in its place. The grafts used varied between 1.5 and 4 cm. in length. The venous grafts, and to a lesser extent the arterial grafts, were usually somewhat greater in diameter than the re-

Table 1 Animals Living with Vein Grafts

DOG	SURGERY	POSTOPERATIVE FEMORAL PULSES	AORTOGRAM
54–57	2/24/54	Strong throughout course	Graft patent 10/1/54
54–62	3/12/54	Strong except for diminution in 2nd and 3rd postoperative weeks	Graft patent 10/1/54
54–70	3/25/54	Strong except for absence in 2nd and 3rd postoperative weeks	Graft patent 10/5/54
54–93	4/2/54	Strong throughout course	Graft patent 10/5/54
54–94	4/16/54	Strong throughout course	Graft patent 10/7/54
54–100	5/5/54	Strong except for diminution in 2nd postoperative month	Graft patent 10/12/54
54–129	5/21/54	Strong for 2 weeks postoperative. Diminution after that time	Graft patent 10/12/54
54–239	8/18/54	Strong throughout course	Not done
54–268	9/8/54	Strong throughout course	Not done
54–270	9/8/54	Strong throughout course	Not done

cipient aorta. An everting mattress suture of 5-0 arterial silk interrupted in two places was used in all suture lines After removal of the Potts clamps and before abdominal closure, a segment of the polyvinyl formal sponge was sutured snugly around the graft and the adjacent aorta No antibiotics or anticoagulants were used during or after surgery.

Postmortem examination of all animals dying after operation was carried out to ascertain the status of the graft, and some of the animals were sacrificed at varying intervals postoperatively for the same purpose. In some instances aortograms were done for postoperative study of the graft

RESULTS

Of the 43 dogs subjected to surgery, 6 were rejected for analysis because of technical factors not bearing on the method. Two animals, one with an arterial and one with a venous graft, had patent grafts after surgery, but complete thrombosis developed within 24 hours At autopsy no error in technique was found to explain this, and these two experiments probably represent inherent difficulties with this method of grafting. Although a technical error at surgery cannot be entirely excluded, these animals are included in the study as acceptable subjects. The remaining group, all of

Table 2 Animals Dead Following Vein Grafting

DOG	SURGERY	POSTOPERATIVE COURSE	DEATH	AUTOPSY FINDINGS
54–5	1/22/54	Femoral pulses strong through postoperative period. Routine sacrifice	3/17/54	Graft patent with slight dilatation. Wall thin, smooth
54–11	2/5/54	Femoral pulses strong through postoperative period. Routine sacrifice	4/12/54	Graft patent with no dilatation but some shortening. Wall smooth
54–61*	3/3/54	Femoral pulses strong through postoperative period. Routine sacrifice	7/14/54	Graft patent; a moderate fusiform aneurysm with thin, smooth wall
54–64	3/10/54	Femoral pulses weak in first and second postoperative weeks, with later improvement. Routine sacrifice	5/26/54	Graft patent with slight fusiform dilatation. Wall in central portion quite thin and ballooned
54–109	4/21/54	Dog paraplegic with absent femoral pulses 4/22 Sacrificed 4/23/54	4/23/54	Graft looked necrotic. Filled with thrombus
54–208	7/13/54	Femoral pulses strong through postoperative period. Developed distemper and died	7/19/54	Graft patent with thin mural thrombus. Graft not related to death
54–207	*7/15/54*	*Femoral pulses strong for 2 days and fair after 1* week. Sudden paraplegia and death at 2 weeks	*7/29/54*	*Graft filled with thrombus and ruptured near* proximal suture line
54–225	8/11/54	Femoral pulses diminished 8/12 and absent 8/13. Sacrificed 8/13/54	8/13/54	Graft occluded with thrombus, no obvious cause for this
54–127	8/27/54	Femoral pulses present immediately after surgery but absent from first day until sacrifice	10/1/54	Graft filled with thrombus without obvious cause. Lumen narrow at suture lines
54–254	8/31/54	Femoral pulses strong through 9/8. Found dead 9/9/54	9/9/54	Abdomen filled with clotted blood. Graft wall weak and split open in several places

* Human saphenous vein with thickened, diseased wall used.

which had palpable femoral pulses for at least 24 hours after surgery, consisted of 19 dogs with venous grafts and 16 with arterial grafts.

At the time of this report 10 of the animals with venous grafts are alive at intervals varying from 6 weeks to 8 months postoperatively. The results in these animals are summarized in Table 1, which shows a patent, functional graft in all 10. Aortograms in the five animals followed for the longest time since surgery showed a similar picture in each instance. There was evidence of a 15 to 25 per cent decrease in the size of the lumen at one or both anastomotic lines despite the everting suture employed. The graft itself appeared tortuous and somewhat dilated but in no case had the dilatation reached aneurysmal proportions at the time of aortography

In addition, 10 dogs with venous grafts have undergone postmortem examination, and the experiments in these animals are summarized in Table 2 In four dogs routine sacrifice was carried out while the animal was in good condition, and death in one was due to distemper, the graft being

Table 3 Animals Living with Arterial Grafts

DOG	SURGERY	POSTOPERATIVE FEMORAL PULSES	AORTOGRAM
54–92	4/14/54	Strong throughout course	Graft patent 10/7/54
54–97	4/23/54	Slight diminution in 1st 4 months postoperative, strong later	Graft patent 10/8/54
54–118	4/30/54	Strong throughout course	Graft patent 10/8/54
54–159	5/28/54	Strong except for diminution from 3rd through 8th postoperative week	Graft not patent 10/12/54. Femoral pulses through collaterals
54–196	7/2/54	Strong throughout course	Not done
54–200	7/8/54	Strong throughout course	Not done
54–218	8/6/54	Strong for 3 weeks postoperative, then disappeared with slow return	Graft patent 10/13/54
54–230	8/13/54	Diminished during 1st week with gradual disappearance and weak return	Graft not patent 10/13/54. Femoral pulses through collaterals
54–253	9/1/54	Strong throughout course	Not done

intact. Several of these animals showed slight dilatation of the graft, but the only true aneurysm occurred in the dog in which a diseased, human saphenous vein was used At sacrifice 4 months after operation this graft had enlarged approximately three times in diameter in a symmetrical, fusiform manner. The encasing plastic sponge had thinned as the vein enlarged. The remaining five animals died or were sacrificed with evidence of difficulties related directly to the procedure Graft rupture had occurred in one, thrombosis and rupture in a second, and complete thrombosis in the remaining three In one of the dogs with thrombosis, the graft wall appeared grossly necrotic 48 hours after surgery.

Of the dogs with arterial grafts 9 are alive at intervals varying from 7 weeks to 6 months postoperatively. In Table 3 are presented the data from this group, showing a patent, functional graft in 7. Aortograms in the three animals 6 months after surgery showed a different picture from the aortograms in the venous graft animals Although there was constriction at the anastomotic line and some irregularity of the graft outline, the graft itself did not appear dilated in any case

Postmortem examination has been done on 8 dogs with arterial grafts, and these experiments are summarized in Table 4. One animal was sacrificed 2 months postoperatively while in good condition. The graft in this case was not dilated but a large mural thrombus filled one side of the lumen. The other 7 animals all showed evidence of trouble related to the operation prior to death or sacrifice. In three of these the graft thrombosed, in two it ruptured, and in two a false aneurysm formed with thrombosis in one and secondary rupture in the other. The secondary rupture occurred almost three months postoperatively in the one graft placed in the thoracic aorta.

Examination of microscopic sections from both venous and arterial grafts 2 to 4 months postoperatively showed considerable fibrous tissue reaction in the interstices of the plastic sponge. Little or no foreign body reaction was evident in and around the sponge. The walls of the venous grafts were represented by a relatively acellular tube of fibrous tissue of varying thickness. The walls of the arterial grafts showed a persistence of elastic fibers, but these fibers were markedly swollen and hyalinized. Nuclei in the media were either absent or pyknotic. The spaces between elastic fibers were widened and frequently filled with red blood cells. It was difficult to identify an intimal layer in grafts of either type. In most specimens a small organized mural thrombus was present. Study of longitudinal microscopic sections at the anastomotic line suggested a thin endothelial layer growing from the host vessel into the graft.

DISCUSSION

The follow-up period in this experiment is still too short to determine the ultimate fate of the grafts. Although some of the grafts have functioned satisfactorily to date, a significant percentage of early failures was encountered with this technique. Thus, early thrombosis or rupture occurred in about 25 per cent of the animals with venous grafts and in approximately 50 per cent of those with arterial grafts. Most commonly, the first signs of trouble became evident in the second or third week after operation. In some cases, later proved by aortogram to have patent grafts, the diminution in femoral pulsations was transitory but in others it remained permanently. This percentage of failure is higher than that following use of homografts in similar experiments in this laboratory.

Although the early results would indicate a superiority of the venous graft under the conditions of this experiment, study of the aortograms made at 6 months suggests that the later results with arterial grafts may be more satisfactory. The vein segments at this time interval showed tortuosity and slight dilatation which were absent in the arterial grafts, and it appeared that aneurysmal dilatation was beginning despite the surrounding plastic sponge. In the one experiment in which a human saphenous vein was used, it was shown at autopsy that thinning and enlargement of the sponge could occur despite the fibrosis occurring through its interstices. Other cases showed graft rupture, both early and late, despite the sponge.

In many experimental and clinical methods of graft preservation an attempt is made to reduce host specificity of the graft by devitalizing it before its use. In the present experiment, where this was not done, several of the reported failures might possibly be due to incompatibility of donor and recipient. This interpretation might be placed on the three experiments in

Table 4 Animals Dead Following Arterial Grafting

DOG	SURGERY	POSTOPERATIVE COURSE	DEATH	AUTOPSY FINDINGS
54–14	2/3/54	Femoral pulses strong through postoperative period Routine sacrifice	4/12/54	Graft patent, no dilatation 40% of lumen filled with endothelialized mural thrombus
54–38	2/10/54	Graft transmitted pulsation at operation but postoperative femoral pulses absent until dog sacrificed	2/19/54	Graft filled with non-adherent thrombus; no evident cause for this
54–27	3/18/54	Femoral pulsations strong for 1 week but then absent for 2 weeks with gradual return to strong pulsation Aortogram 9/29/54 showed occluded graft. Sacrificed	9/30/54	Graft filled with organized thrombus Femorals reconstituted through large collateral channels
54–83	3/31/54	Femoral pulsations strong for 1 week but then disappeared Sacrificed	4/9/54	Graft filled with thrombus, wall friable
54–145*	5/13/54	Femoral pulsations strong for 12 weeks. Animal then died overnight	8/5/54	Graft ruptured through small area in its midportion with false aneurysm and later secondary rupture, fatal hemorrhage
54–141	5/14/54	Femoral pulsations strong for 1 week but then disappeared Slowly developed paraplegia Sacrificed	6/16/54	Graft replaced by large clotted false aneurysm with only a few remnants of graft wall remaining
54–247	8/24/54	Femoral pulsations strong for 4 days Found dead 8/29/54	8/29/54	Graft intact except for small rupture just proximal to distal suture line Fatal hemorrhage
54–251	8/25/54	Femoral pulsations strong for 1 week Animal weak at 10 days and dead at 2 weeks	9/7/54	Disruption of proximal suture line with fatal hemorrhage. Graft otherwise intact.

* Graft placed in descending thoracic aorta under hypothermia.

which unexplained thrombosis occurred in the first 24 hours. In one of these three the entire graft appeared necrotic 48 hours after surgery.

These studies suggest that the use of heterografts in the human aorta should be recommended only with considerable reservation until further experimental work can be evaluated.

SUMMARY AND CONCLUSIONS

Grafts from the inferior vena cava and the aorta of pigs were used to substitute for segments of the aorta in dogs. In an attempt to prevent aneurysm formation, the grafts were supported by an inert plastic sponge. These experiments showed an early failure rate of 25 per cent using venous grafts and of 50 per cent using arterial grafts. Early failures were due to thrombosis or rupture, either at the anastomotic line or in the middle of the graft. In only one case was significant aneurysm formation demonstrated, but examination of aortograms taken at 6 months suggested that the venous grafts may develop aneurysms at a later date.

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PLASTIC VENOUS PROSTHESES*

RICHARD H. EGDAHL, DAVID M. HUME, AND HENRY A. SCHLANG**

Search for the ideal blood vessel graft continues. In arteries, venous autografts with or without fascia or pericardial backing, arterial homografts and plastic cloths[1] are used at present with considerable success. Venous autografts have usually been used to replace resected or damaged venous segments, with variable results. The incidence of thrombosis is high in venous system grafts, and often the graft becomes occluded by scar tissue in the postoperative period. With a widening surgical horizon, there will undoubtedly be an increasing number of resections of the portal vein for cancer, bilateral jugular vein resection in the older age group, and reconstructive procedures in the superior vena cava syndrome. Because of the technical difficulties and questionable successes of some of these procedures, it was felt desirable to study possible alternatives in the venous grafting procedure.

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** The opinions or assertions contained herein are the private ones of the writers and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

In 1942 Blakemore and associates[2] re-introduced the non-suture method of anastomosis as applied to arteries, using vitallium for the prosthetic material They also performed a series of portacaval shunts with the non-suture method of anastomosis, but results were equivocal and the work was not continued. One purpose of this study is to evaluate the non-suture method of anastomosis as applied to veins in various areas Prostheses were placed in the venous rather than in the arterial system to give a much more severe test of the anastomotic procedure. The second purpose of this study is to determine the applicability of plastic cloth as a venous graft material.

METHODS

Healthy adult mongrel dogs ranging in weight from 15 to 27 kilograms were used for all surgical procedures. Intravenous Nembutal, 30 mg. per kilogram, was chosen as the anesthetic agent. All operations were performed under sterile conditions, and antibiotics were not given. The first part of the experiment was set up to determine the most effective method of using polyethylene as a venous prosthesis. For this purpose, 3 groups of dogs were contrasted In the first group, pieces of polyethylene tubing 2 cm. in length and coated with Dri-Film (GE SC-87) were inserted into the external jugular vein The ends of the polyethylene were slightly flared with heat to prevent slipping of the tie, and a 1 cm. segment of the vein was resected before the prosthesis was put in place Two sizes of tubing were used, depending on the diameter of the vein One size of tubing was 3 99 mm. inside diameter, and 5 95 mm. outside diameter, the other 5 28 mm. inside diameter and 6 86 mm. outside diameter. The flared tubes fit easily into the severed vein end, and 3-0 cotton ties held the venous prostheses securely in position.

In the second and third groups, a graft was taken from the right external jugular vein and placed inside a 2 cm. length of polyethylene tubing, whose ends had been slightly flared with heat. The ends of the vein graft were turned back over the tubing and tied, and the vein-lined prosthesis was tied in the left external jugular vein Solid polyethylene tubing was used in group 2 and polyethylene tubing with 6 perforations, each 2 mm in diameter, in group 3 The only difference between the two groups was the presence of perforations in the tubing of group 3. Care was taken to remove the trapped air between the [illegible] l tubes.

Group 4 was identical to g[illegible] is 6 cm in length and had 18 perfo[illegible] 1). In group 5, 2 solid polyethylene tubes ½ cm in length carried 2 cm external jugular grafts, thereby leaving the central portion of the graft unsupported. Group 6 evaluated perforated polyethylene vein-lined prostheses in the portal circuit Either straight prostheses 2 cm in length were inserted into the portal vein with the splenic vein ligated, or curved prostheses were inserted between the superior mesenteric and left renal veins. Average time for insertion of the prostheses was 5 to 10 minutes

The second part of the experiment utilized plain white, tightly-woven, 100 per cent orlon cloth as a venous graft material. At operation, sterile cloth was fashioned into grafts of the proper diameter by sewing the folded edge with 5-0 silk suture. A segment of vein approximately 1.5 centimeters in length was resected and the grafts sutured by everting anastomosis into the defect thus created. Orlon cloth grafts were placed in the external

jugular, portal, and inferior vena cava veins. There was little bleeding and good postoperative flow in all instances.

Serial venograms with 35 per cent Diodrast were performed in representative experiments with both polyethylene tubing and orlon. Vascularization of the vein graft lining of the perforated polyethylene tubes was visualized by clamping the vein above and below the prosthesis in situ,



Fig 1. Six centimeter perforated vein-lined polyethylene tube in situ at completion of operation

and injecting 10 cc. of 1:8 india ink into the common carotid artery on the side of the prosthesis Microscopic sections were made of the tissue which had grown through the perforations of the perforated prostheses

RESULTS

Table 1 outlines results with the first five groups. All siliconed tubes were thrombosed at necropsy, with an average follow-up of 25 days. Venograms on the first postoperative day in two instances revealed almost complete thrombosis.

Table 1. Summary of Results with Various Types of Polyethylene Venous Prostheses

	EXCELLENT*	SATISFACTORY	UNSATISFACTORY	THROMBOSIS
2 cm siliconed				10 (100%)
2 cm vein-lined solid	1 (6%)	2 (12%)	2 (12%)	12 (70%)
2 cm vein-lined perforated	14 (61%)	4 (17%)	2 (9%)	3 (13%)
6 cm vein-lined perforated	6 (38%)	2 (12%)		8 (50%)
2 cm vein-lined 2-tube	9 (69%)	1 (8%)	1 (8%)	2 (15%)

* Excellent Good blood flow, smooth graft [illegible]
Satisfactory [illegible]
Unsatisfact[illegible]
Thrombosis [illegible]

External Jugular Prostheses. Groups 2 and 3 had an identical average follow-up period of 20 days. Table 2 breaks down the group into specific time intervals, and it can be seen that during any interval the perforated vein-lined prostheses demonstrated marked superiority over the solid vein-

Table 2 Comparison of 2 cm. Solid with 2 cm. Perforated Vein-lined Polyethylene Prostheses

INTERVAL FROM SURGERY TO NECROPSY	2 CM SOLID				2 CM PERFORATED			
	EXCELLENT*	SATISFACTORY	UNSATISFACTORY	THROMBOSIS	EXCELLENT	SATISFACTORY	UNSATISFACTORY	THROMBOSIS
5–7 days		1			1			
7–13 days	1			3	4	1	1	
14–21 days		1	1	8	4	3	1	
22–50 days			1	1	4	1	1	2

* See footnote to Table 1

lined prostheses. Seventy-eight per cent of the perforated prostheses were either excellent or satisfactory, in contrast to only 12 per cent for the solid prostheses. There were often discoloration and maceration of the vein graft of the solid tubes at necropsy, even in those in which partial patency was

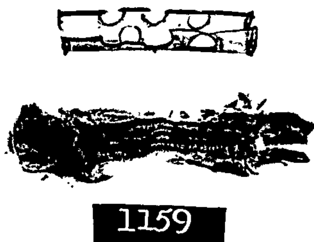


Fig. 2 Two centimeter perforated vein-lined polyethylene tube from external jugular vein of dog, 26 days after insertion

present. These latter findings were not observed in the case of vein grafts from perforated prostheses (Fig. 2). Fibrosis of the vein graft appeared to be a very gradual and not invariable occurrence, for vein grafts from patent perforated prostheses 42 and 45 days after insertion were unaffected

India ink injections demonstrated the adequacy of vascularity through the perforations of the prostheses, for with the tube segment clamped from the venous circulation, carotid artery injections resulted in marked intimal staining. In all cases of perforated prostheses, bridging units of tissue were observed going from the peri-tubal area through the perforations to the graft. Microscopic examination revealed numerous capillaries and young blood vessels in this bridging tissue. The intima of the grafts of those prostheses which remained patent healed well to the intima of the vein into which the prosthesis was inserted.

Fifty per cent of the 6 cm. vein-lined perforated prostheses of group 4 were either excellent or satisfactory after an average follow-up period of 18 days. Vein grafts from these tubes were of good color and 10 to 15 per cent of them had noticeable fibrosis. One 6 cm. perforated prosthesis was followed for 43 days, at which time there was little or no constriction of the graft and excellent flow. India ink injection studies indicated the presence of abundant vascularization of the vein graft through the perforations. With the use of two short tubes and a 2 cm. vein graft in the non-suture method of anastomosis, results were approximately the same as with 2 cm. perforated prostheses. There was some fibrosis of the vein graft in some tubes, but none, for example, in the longest follow-up of 51 days.

Portal System Prostheses. Group 6 was made up of a series of 14 abdominal non-suture venous anastomoses. Of nine cases of superior mesenteric-renal vein anastomosis, eight were followed for an average time of 20 days. One case died on the first postoperative day of hemorrhagic pneumonia, but the prosthesis was patent and the bowel of normal color. Three of the eight cases revealed patent prostheses at 13, 16, and 29 days, respectively. There was moderate fibrosis and thickening of the vein graft in these three cases. The remaining five cases had solidly thrombosed vein grafts in the prostheses, but normal appearing bowel and obvious venous collateral circulation of the superior mesenteric vein were present. Serial venograms in one of these cases demonstrated patency until the seventh day. One portacaval anastomosis was patent at 14 days, but there was moderate thickening of the vein graft. Of four cases with portal vein prostheses, three were widely patent at 8, 14, and 14 days. The fourth dog died on the second postoperative day and at necropsy dark bowel and a thrombosed tube were found.

Studies on the use of orlon cloth as a venous grafting material gave almost uniformly negative results. Nine orlon grafts in the external jugular vein were thrombosed at time of necropsy, ranging from 1 to 49 days. Venograms in two instances demonstrated partial thrombosis as early as one and two hours after operation. Of nine orlon grafts in the inferior vena cava, eight were thrombosed in less than 21 days, and one was patent at six days. The patent graft had much fibrin on the intimal surface of the cloth, and there was definite reduction in lumen. There were eight orlon grafts placed in the portal vein. Four dogs failed to recover from the operation, despite adequate flow when they were closed. At necropsy the bowels were dark and in three of the four cases the orlon grafts were thrombosed. In one of the four cases, the orlon graft was widely patent. It was noted in this dog that, despite clamping of the portal vein for only 25 *minutes* during the

anastomosis, the bowels failed to regain their color to any extent following placement of the graft. Of four survivors, three had normal appearing bowel, much portal collateral circulation and solidly thrombosed orlon grafts at 25, 26, and 31 days. The fourth prosthesis was widely patent at 11 days, with good flow and bowel color.

DISCUSSION

The superiority of perforated over solid vein-lined polyethylene prostheses used in the non-suture method of anastomosis in veins is clearly shown by this study The importance of external vascularization of arterial homografts has previously been noted by several investigators.[3,4] Our work demonstrates that there are numerous blood vessels in the tissue bridging through the perforations. Discoloration of the central portion of the vein grafts of solid tubes was in marked contrast to the uniformly normal color of the grafts in perforated tubes. These facts point to the necessity for an adequate vascular bed around venous grafts, and emphasize the desirability of using perforated instead of solid prostheses in the non-suture method of anastomosis

The incidence of good results falls with increasing length of venous prosthesis The most likely explanation for this is that there is a longer area of constriction in the venous flow Polyethylene tubing, while ideal from the standpoint of availability, minimal tissue reaction and workability, has the disadvantage of having relatively thick walls as manufactured commercially. This means that polyethylene tubing lined with a vein graft presents a vascular channel of quite markedly diminished size compared to the vessel into which it is put It seems certain that the use of thinner walled prostheses, perhaps made of vitallium, stainless steel, or irradiated thin polyethylene tubing, would result in a higher incidence of patency than we have encountered However, it has been demonstrated that polyethylene as we used it will give a high percentage of satisfactory results.

Ligation of the superior mesenteric or portal vein results in death in 100 per cent of dogs if they are not given antibiotics[5] We had only one death in 14 cases of portal or superior mesenteric-renal anastomosis with perforated vein-lined prostheses However, about 50 per cent of the vein grafts were thrombosed at necropsy, and there was abundant collateral circulation. Furthermore, more fibrosis and constriction of the vein grafts in the prostheses were noted in these tubes than in those inserted in the external jugular vein It is not possible, on the basis of this work, to recommend the non-suture method of anastomosis in portacaval shunts or similar procedures. However, the greater caliber of human vessels and use of thinner walled prostheses may make the procedure practicable.

Orlon cloth was found to be an unsatisfactory venous graft material, with only an occasional patent graft in a short follow-up period.

CONCLUSIONS

1. Perforated polyethylene vein-lined tubing may be used successfully in the non-suture method of anastomosis in veins.

2. Perforated prostheses are superior to solid prostheses because vascularization to the vein graft of the former can occur through the perforations.

3. Orlon cloth appears to be an unsatisfactory venous grafting material.

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OBSERVATIONS ON THE NATURAL HISTORY OF RENAL HOMOTRANSPLANTS IN DOGS*

JOSEPH E. MURRAY, STANLEY LANG, AND BENJAMIN F. MILLER

Renal homotransplants in dogs follow a relatively constant course. They function immediately and survive for from one to eight days, at which time rejection occurs rather explosively.[1, 2] In humans the homotransplanted kidney passes through a seven to twenty day period of oliguria or anuria before resuming function. The excretion of urine may then persist for months and cease only with the death of the patient or with secondary infection [3]

In an effort to determine whether or not these differences are species specific, laboratory conditions were altered to simulate those involved in human homotransplantation. The human recipient naturally has impaired renal function Moreover, there is always some unavoidable period of ischemia of the donor kidney prior to its revascularization, usually about 2 to 4 hours. Other variables possibly affecting the transplant's function are the previous functional state of the donor kidney, problems associated with blood compatibility, and the varying sites of implantation.

The usual dog transplants have been performed with a normal donor kidney implanted into a healthy recipient after a 15 to 20 minute period of ischemia, the neck, groin, or renal fossa being the usual sites for implantation

METHODS

In this study we varied two factors. The donor kidney was made ischemic prior to revascularization, and the renal function of the recipient was impaired.

Preliminary work of our own had suggested that ischemia of the donor kidney did affect the course of the transplant. However, with one normal kidney still remaining, the output was too low to be useful for quantitative

* From the Departments of Surgery and Medicine, Harvard Medical School, and the Peter Bent Brigham Hospital, Boston This study was aided by Grants from the Bristol Myers Pharmaceutical Co, The Lasdon Foundation, the National Institute of Health, and Eli Lilly and Co.

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In this study we varied two factors. The donor kidney was made ischemic prior to revascularization, and the renal function of the recipient was impaired.

Preliminary work of our own had suggested that ischemia of the donor kidney did affect the course of the transplant. However, with one normal kidney still remaining, the output was too low to be useful for quantitative

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evaluation The period of ischemia was obtained by allowing the donor kidney to be exposed to room temperature wrapped in a saline sponge for two to four hours. In some of the experiments the donor animal was sacrificed and the kidneys allowed to remain in situ during the selected period of time

Trying to simulate the uremic state in a dog is difficult, especially if the experiment requires survival of the dog for periods longer than 7 days. A bilaterally nephrectomized dog will die within a week and thus not allow time to evaluate the end result of the homograft. Unilateral nephrectomy followed by partial nephrectomy of the remaining kidney after permanent ligation of half of its blood supply produced unpredictable results. The method selected to produce an elevated blood urea nitrogen (BUN) with sufficient renal tissue left for survival is based on the work of Van Slyke, who temporarily impaired renal function by producing transient ischemia of the kidney.[4]

At the first operation the urinary bladder is bisected and cystotomy tubes inserted into each half-segment to allow for divided urine collection.[5] The left renal artery is occluded at this time for 2 hours, the period of time found optimal to obtain maximum renal damage consistent with subsequent regeneration Following this operatior ... e the
left undergoes a predictable patter ... in 7
or 10 days by return of function, ... eenth
to twenty-first day.

The second operation is timed about 7 days later. The kidney is removed, the "ischemic" homograft inserted into the right iliac fossa and the ureter put into the right half of the bladder. The dog immediately runs an elevated BUN which lasts for 10 to 14 days, depending on the rapidity of the left
... f survival with impaired renal
... through its full course. There
... pe of impaired renal function
with clinical uremia.

Prior to use of this technique secondary infection and technical difficulties in urine collection were frequent. As a result of infection some of the recipients planned as normal became "uremic" and are included in the "uremic" series if sufficient data on the experiment are available.

RESULTS

An illustrative experiment after the bladder hemisection and the 2-hour occlusion of the left renal artery shows that the urine output from this side practically ceases for 8 days while the right kidney compensates with an increased output
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The volume excreted by the transplant into such a "uremic" recipient does increase enough to give quantitative differences under varying conditions There was no prolongation of the survival period but the pattern of excretion changed considerably There were peak days of excretion, usually the third to the fifth days. Some kidneys started the peak excretion on the first day, others started slowly and gradually increased. The 4-hour ischemic

donors seemed to be slower in starting their excretion than the 2-hour ischemic kidneys

Most of the animals were ill, weak, and not eating well at the time of their sacrifice. However, they did not die prematurely and were sacrificed at the selected times for study.

Two interesting variations in the length of survival are noted. The kidney from the one successful neonatal donor (22-day-old puppy with 2-hour ischemia) functioned 12 days and was still functioning at time of sacrifice. Because the left kidney did not function in this dog owing to infection, hemodialysis on the "artificial" kidney on the fifth day helped keep it alive. However, the transplant was not life-sustaining for the animal became moribund by the twelfth day. At autopsy the transplanted kidney was perfectly reactionless grossly and there was clear urine in the bladder.

The second long survival of function was in a pregnant recipient. Here the volume was never high, but on the twentieth day urine was still coming, and at autopsy urine was noted in the bladder. This animal, an early one in the series, had a left ureterostomy which became infected, necessitating sacrifice.

In summary, the 2-hour ischemic donor kidney put into normal dogs had no peak excretion days but had constant low daily outputs till the day of rejection. The same type of kidney put into "uremic" recipients had greater volumes and reached a peak usually on the third or fifth day. Dog 125, the only one failing to show this peak, had such rapid regeneration of her left kidney that it never was "uremic." The neonatal kidney had a slow rise to the peak excretion day and functioned for 12 days.

The 4-hour ischemic donor kidney formed a more definite pattern of delayed excretion, rising to the peak on the fifth and sixth days. The pregnant recipient ran a long survival course, which of course invites speculation regarding this point. Most of our other pregnant recipients aborted so we have no accurate data on this point.

The urine secreted by the transplant was usually pale yellow, slightly cloudy, with a specific gravity of 1.004 to 1.014 (normal range for dog goes up to 1.040 or 1.050), and a pH of 5.0 → 6.5 for the first few days. As it diminished in amount the color darkened, protein appeared and at cessation of function it was usually blood tinged.

SUMMARY AND CONCLUSIONS

No definite conclusion can be made. Several observations do seem valid, however. Under the conditions of the experiment the survival time of the transplant is not prolonged. The neonatal donor kidney and the pregnant recipient both persisted longer than any of the others, but neither was life-sustaining even though still functioning at the time of sacrifice.

The pattern of daily excretion definitely seemed altered in rather consistent fashion both by making the recipient "uremic" and by increasing the period of ischemia of the donor kidney. In the four-hour ischemic donor kidney the altered renal excretion pattern did tend to resemble that of the human in that the excretion was delayed and only slowly rose to a peak.

There is some evidence that the "explosive" nature of the rejection of the dog homotransplant can be altered, for in some of these experiments the kidney, though obviously failing, was still functioning to some degree.

The chief value of this work thus far lies in the evidence that we can alter in a moderately reproducible fashion the excretion rate of a transplant. Such environmental factors should be considered with genetic and immunologic ones in assaying the over-all status of any particular transplant.

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THE STERILIZATION OF HUMAN ARTERIAL HOMOGRAFTS WITH BETA-PROPIOLACTONE*

Experimental and Clinical Observations

D. EMERICK SZILAGYI, PAUL R. OVERHULSE, CLAIBOURNE P. SHONNARD, AND GERALD A. LOGRIPPO

In the past few years, with the widening scope of vascular surgery, the demand for human vascular grafts has markedly increased. This greater demand has multiplied the difficulties of obtaining grafts. Perhaps the greatest of these difficulties is the cumbersome technique, with its elaborate preparations and time wasting details, that one is obliged to employ in order to procure grafts in an aseptic manner. It is readily seen that if one could remove arterial segments in the course of a routine autopsy without regard to asepsis and sterilize them later, the procurement of grafts for blood vessel banks would be immensely facilitated. The importance of a simple and safe method of sterilizing human arterial blood vessel material is thus obvious. Among the physical[1-4] and chemical means of achieving this end proposed in the past, sterilization with ethylene oxide[5] has been found by some to be satisfactory in rather extensive clinical trials. The handling of ethylene oxide, however, is cumbersome and dangerous owing to the volatility and explosive qualities of this compound.[6, 7]

In this study we wish to report some observations on the use of the organic compound beta-propiolactone (BPL) as a sterilizing agent for human arterial grafts. Our attention has been directed to the promising qualities of this substance by Dr. Frank W. Hartman, who had found it to be capable of inactivating a wide range of viruses seeded in plasma and whole blood at drug concentrations that were not deleterious to the plasma proteins[8] Prior to our investigation, Trafas, LoGrippo et al.[9, 10] showed that BPL

* From the Departments of Surgery and Pathology, Henry Ford Hospital, Detroit, Mich.

effectively sterilized contaminated canine arterial transplants and that the rate of success with such grafts was the same as with control specimens.

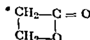
Beta-propiolactone,* an ester of hydroxy-propionic acid, is a stable liquid in its concentrated form but highly unstable when dissolved in water. The lactone ring is capable of opening at either the alkyl or the acyl oxygen bond and it reacts readily with SH, COOH, OH, phenolic or amino groups.

EXPERIMENTAL METHODS AND RESULTS

We sought to determine (1) the lowest concentration of the drug that would, during the shortest contact, effectively sterilize human arterial segments obtained without asepsis at routine autopsies, and (2) whether this concentration of the drug would adversely affect the histologic structure and tensile strength of the grafts, qualities that have cardinal importance in the evaluation of the suitability of a graft for surgical use. Using contaminated specimens from autopsied cadavers, concentrations of 0.5, 0.75, and 1.0 per cent were tried in various combinations with durations of contact of one, two and three hours. A concentration of 0.75 per cent by weight during a contact of two hours was found to assure sterilization in every instance. As an added margin of safety a 1 per cent strength was chosen for the further experiments and possible clinical trial. For the study of the effects of sterilization on histologic structure and physical properties, the arterial segments were treated as follows: The grafts were obtained at autopsies with aseptic precautions no later than eight hours after death. The specimens were washed in saline and the saline wash cultured. The specimens were then divided and the segments to serve as controls were placed in Hanks' solution. The experimental segments were likewise placed in Hanks' solution but to this solution were also added 5 mg. of phenol red per 100 ml. and 0.88 gm. of BPL per ml. The flasks were left in a water bath of 37° C. for two hours. The *p*H of the flasks containing BPL was maintained at 7.0 to 7.4 by the frequent addition of small amounts of N/10 NaOH solution. At the end of two hours the graft was removed from the BPL solution under an ultraviolet hood and transferred to the storage fluid of Hanks' solution. The sterility of the storage fluid of all flasks was tested at intervals of 24 hours and 6 weeks. At intervals of 24 hours, 1 week, 4 weeks and 6 weeks, paraffin sections were made of the treated and untreated specimens and stained with hematoxylin-eosin and Weigert's elastica stain. These sections of treated specimens showed excellent staining qualities and essentially normal histologic structure (Fig. 1). Indeed, at times, the histologic sections of the controlled specimens were impossible to be distinguished from the sections of treated specimens.

Similarly stored treated and untreated segments of artery were subjected to tensile strain by graduated loads of weight. The treated segments showed a slight decrease of elasticity, which never exceeded 15 per cent and averaged 9 per cent These differences in clinical use may be regarded as negligible. The tensile strength of sterilized specimens was not perceptibly altered (Fig. 2).

We were interested in the effects of BPL on the viability of the grafts but were unable to obtain clear-cut results in tissue cultures. It should be

*


pointed out, however, that tissue-culture viability is not a characteristic of great importance for the surgical usefulness of a graft.

CLINICAL OBSERVATIONS

During the past thirteen months homografts sterilized with beta-propiolactone have been used in thirty-one vascular operations

Method of Processing the Grafts. The graft specimens were obtained in

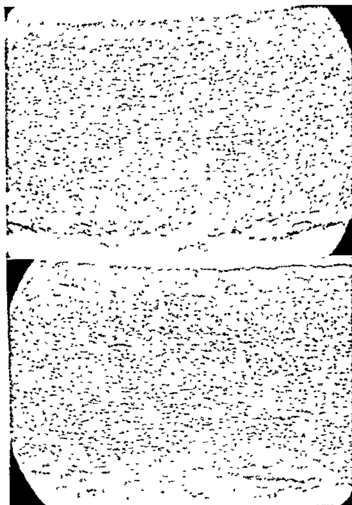


Fig. 1. Histologic section of (*upper*) untreated and (*lower*) treated human arterial segment (aorta) Hematoxylin-eosin stain (×35)

the autopsy room without aseptic technique after the pathologist had removed all * As a rule, the entire aorta from the as the common iliac arteries and th he ligament of Poupart and the floor of the pelvis, were removed. Whenever possible, through special incisions, the femoral arteries were also taken, in continuity with the external iliac arteries. The specimen so obtained was stored in segments, having

* *Special permission was secured for taking the graft, in all instances, from the responsible relative of the deceased.*

been transected at the level of the diaphragm, and, if the femoral arteries were also attached, at the level of the ligament of Poupart bilaterally. After its removal the graft was washed in sterile saline and the saline wash was cultured. The graft was taken to the blood vessel laboratory where the periarterial fat and areolar connective tissue were trimmed away. The stumps of the branches were not ligated until the time of use. The vessel was next placed in a flask containing a saline-bicarbonate solution of the following composition: Isotonic saline (amount depending on the size of the graft; usually 200 to 600 ml.); 0.2 molar sodium bicarbonate (1.68 gm. per 100 ml. of solution), and phenol red (5 mg. per 100 ml. of solution). To the flask was now added a 10 per cent (weight/volume) solution of BPL [obtained by dissolving 0.88 ml of BPL (sp. gr. 1.149) in 10 ml. of cold (4° C.) distilled water] in the amount of 10 cc. per 90 cc. of saline-bicarbonate solution. By vigorous stirring the final solution was brought in

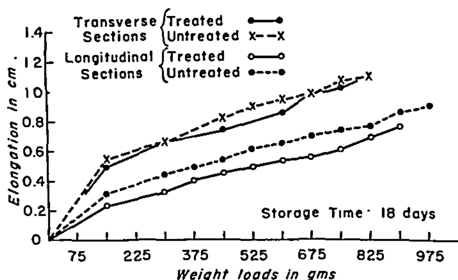


FIG 2. Representative elongation curves of treated and untreated arterial segments (human aorta) The last plot on each curve designates the breakage point.

contact with the entire inside surface of the flask. Neither the phenol red indicator nor the sodium bicarbonate had to be sterilized, since beta-propiolactone rendered them sterile. The flask was placed in a water bath at 37° C. for 2 hours. From this time on, the vessel was handled aseptically. At the end of two hours the graft was taken out of the flask under an ultraviolet hood, washed in a phosphate buffer (pH 7.4) and transferred to a storage flask. Fenwal flasks and Hanks' solution were used for storage. The specimen was refrigerated at 1° to 4° C. After 24 hours a culture of the solution was obtained, and penicillin (200 units/ml.) and streptomycin (1 mg./ml.) were added to minimize subsequent contamination.

At the time of the surgical procedure, when the size and shape of the needed graft had been determined, the suitable graft specimen was brought to the operating room and was transferred to lukewarm saline. After a thorough rinsing in isotonic saline the branches were ligated with fine silk, and the graft was ready for use.

The data with respect to the origin of the graft, together with the bac-

pointed out, however, that tissue-culture viability is not a characteristic of great importance for the surgical usefulness of a graft.

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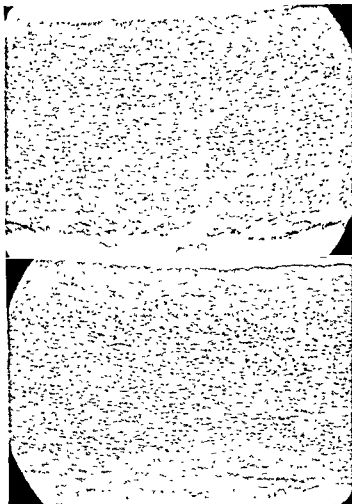


Fig 1. Histologic section of (*upper*) untreated and (*lower*) treated human arterial segment (aorta). Hematoxylin-eosin stain (×35)

the autopsy room without aseptic technique after the pathologist had removed all other thoracic and abdominal organs.* As a rule, the entire aorta from the as the common iliac arteries and th he ligament of Poupart and the floor of the pelvis, were removed. Whenever possible, through special incisions, the femoral arteries were also taken, in continuity with the external iliac arteries The specimen so obtained was stored in segments, having

* Special permission was secured for taking the graft, in all instances, from the responsible relative of the deceased.

teriologic findings before sterilization, the length of storage, and the anatomic location and size of the replaced segments, are listed in Table 1. Most of the donors were 20 to 40 years of age but occasionally satisfactory grafts were obtained from patients who died in the fifth decade of life. The cause of death of the donor had no bearing on the usefulness of the graft provided the physical qualities of the blood vessels were satisfactory. When the cadaver was refrigerated immediately after death, a lapse of time of eighteen or even twenty-four hours between the time of death and the time of obtaining the graft was not deleterious to the usability of the graft. A wide variety of bacterial growth was obtained on the saline wash of the unsterile arterial specimens. All the cultures following treatment with BPL were, however, negative. The grafts were stored in Hanks' solution for practical reasons too lengthy to be discussed. Present evidence is quite convincing that lyophilization of the graft immediately after sterilization would be equally acceptable, or perhaps superior. All but one of the grafts were used within thirty days of the beginning of storage; beyond this interval of time, BPL-treated, wet-preserved arterial grafts appeared to us unsafe.

Results. The essential clinical characteristics of the cases in which the grafts treated with BPL were used are given in Table 2. In what follows only data directly concerned with the assessment of the value of the graft will be discussed.

Certain difficulties in the evaluation of the results must be mentioned although they may appear obvious. The evaluation must, of course, be a short-term one since the longest period of follow-up observation was only 13 months. Patency of the graft is generally regarded as the primary criterion of the short-term results of vascular grafting. When, however, one attempts to measure the usefulness of a particular type of graft—as, in our instance, that of BPL-treated grafts—the application of the incidence of patency as an absolute standard of success is not valid. The success of a grafting operation depends on many factors,* of which the quality of the graft is only one, and probably not the most important one. Unfortunately, however, because of circumstances often encountered in clinical experiments, a control series with untreated sterile grafts that would have reduced the many variables was not possible to develop Comparison of the results with those obtained by other workers by the use of sterile arterial grafts obviously could not correct this deficiency even if reports of such results were available For these reasons the evaluation of the findings must be arbitrary rather than statistical

In speaking of the operative results, distinction must be made between the success of the grafting procedure itself and the clinical outcome of the case. In the large majority of cases a patent graft meant clinical success but at times thrombosis took place distal to a patent graft and clinical improvement failed to follow. (It must be added, however, that in no instance was the clinical course worsened by the grafting operation.)

As shown in Table 2, all the grafts in the *aorto-iliac* region remained patent. In two cases (operations 11 and 18) a segment of artery distal to the graft became occluded by clotting

* Some of these factors may be listed as follows. physiopathologic state of the recipient artery and of the adjacent tissues, systemic blood pressure, regional arterial blood pressure, size of the recipient artery, coagulability of the blood, surgical technique, length of the graft, quality of the graft.

Table 1. Origin, Bacteriology, Length of Storage and Clinical Utilization of Grafts

OP. NO	AGE OF DONOR	CAUSE OF DEATH OF DONOR	HOURS FROM DEATH TO AUTOPSY	BACTERIOLOGY BEFORE STERILIZATION	LENGTH OF STORAGE* (days)	LOCATION AND LENGTH OF SEGMENT REPLACED
1	45	Glioblastoma	3½	No data	4	LSF, 15 cm
2	30	Retroperit. sarcoma	10	*Staph albus* (non-hem), *A aerogenes*	6	AoB
3	30	Retroperit sarcoma	10	*Staph albus* (non-hem), *A aerogenes*	11	RSF, 22½ cm
4	30	Retroperit sarcoma	10	*Staph albus* (non-hem), *A aerogenes*	23	RSF, 7 cm
5	30	Retroperit sarcoma	10	*Staph albus* (non-hem), *A aerogenes*	29	RSF, 11 cm.
6	20	Patent ductus art †	5	*A aerogenes*	12	REI, 12 cm
7	31	Diffuse peritonitis (gastrectomy) †	14	*Staph aureus* (hem)	8	AoB
8	31	Diffuse peritonitis (gastrectomy) †	14	*Staph aureus* (hem)	15	LSF, 27 cm.
9	31	Diffuse peritonitis (gastrectomy) †	14	*Staph aureus* (hem)	20	LSF., 30 cm
10	49	Cor pulmonale	5	*E coli*, *Staph albus* (hem)	10	AoB
11	43	Aortic stenosis	12	*Ps aeruginosa*	11	LCI, 0 cm.
12	38	Lupus erythemat. dissem	9	*Staph albus* (non-hem), *Ps aeruginosa*	11	RSF, 24 cm
13	38	Lupus erythemat dissem.	9	*Staph albus* (non-hem), *Ps aeruginosa*	18	RSF., 32 cm.
14	20	Lymphatic leukemia	5	Yeast and mold	5	AoB
15	20	Lymphatic leukemia	5	Yeast and mold	12	RSF, 29 cm
16	20	Lymphatic leukemia	5	Yeast and mold	14	RSF, 26 cm
17	47	Carcinoma of breast	3	*B subtilis*, *E coli* Enterococcus (hem)	32	AoB.
18	44	Carcinoma of lung	5	*E coli*, Enterococcus (hem)	2	AoB
19	44	Carcinoma of lung	5	*E coli*, Enterococcus (hem)	2	RSF, 28 cm
20	22	Lupus erythemat dissem	11	*E coli*, *B subtilis*	1	RSF, 30 cm.
21	22	Lupus erythemat dissem	11	*E coli*, *B subtilis*	4	AoB
22	30	Mitral insufficiency (commissurotomy) †	2½	No data	8	AoB
23	26	Rh. heart disease	10	Coliform, *Ps. aeruginosa* Strep (hem. & non-hem)	17	AoB.
24	43	Carcinoma of cervix	22	Coliform, Enterococcus (hem.)	43	AoB
25	27	Diffuse peritonitis (hysterectomy)	18	*Staph albus* (hem), coliform, yeast	27	AoB.
26	17	No data, received from outside source	No data	Coliform, *Ps aeruginosa*	25	RSF, 11 cm.
27	46	Spinal cord tumor†	13	Coliform, Strep (non-hem)	25	AoB.
28	31	Carcinoma of breast†	1	Coliform, *Staph albus* (hem)	26	LPo, 20 cm.
29	46	Spinal cord tumor†	13	Coliform, Strep. (non-hem.)	29	LCF, LSF, 40 cm.
30	34	Carcinoma of sigmoid colon	9	Coliform, Strep (non-hem)	2	AoB.
31	30	Malignant hypertension	12	Coliform	23	RCI, 8 cm

Abbreviations AoB, aortic bifurcation; RSF., rt. superficial femoral, LSF, lt superficial femoral, RCF., rt. common femoral, LPo, lt. popliteal artery, LCF, lt common femoral, RCI, rt common iliac, REI, rt external iliac, LCI, lt. common iliac.

* Stored in Hanks' solution at refrigeration temperature (4° C) † Postoperative death

In the *femoro-popliteal* region three grafts out of sixteen became thrombosed in the immediate postoperative period (operations 4, 5 and 20). One remained only partially open (operation 29) owing to distal thrombosis. Another case (severe diffuse arteriosclerosis with impending gangrene of the foot) showed a patent graft for seven months, when thrombosis in the arterial bed distal to the graft led to occlusion of the graft (operation 8).

In two cases the clinical course was terminated by death, unrelated to the grafting operation. In one of these (operation 7, case 6) the patient died suddenly from a massive coronary infarct six weeks postoperatively. In the other case (operation 23, case 19) cardiac arrest occurred three weeks after operation while the femoral region was being explored for control of hemorrhage from the proximal stump of the common femoral artery in which extensive intimectomy had been carried out at the time of the original operation. (The graft was found to be functioning well and appeared intact.)

Since we are not at present concerned with the clinical value of the operations performed, but rather with the usefulness of the grafts employed, we are justified in disregarding complications unrelated to the grafts and in counting every open graft as a good result irrespective of the clinical outcome. In this manner operations 4, 5, 8, 11, 18 and 20 are classified as successful. Likewise, in the two cases of death—on neither of which had the fate of the graft any bearing—the results of the grafting procedures (operations 7 and 23) must be considered satisfactory. Thus, judged by the behavior of the grafts alone, 28 of the 31 operative procedures performed (or 90 per cent) yielded good early results. According to anatomic location but assessed by the same standard, all the aorto-iliac and 82 per cent of the femoro-popliteal grafts were successful. The latter figure is by far the more significant since the behavior of long and narrow segments such as used in the femoro-popliteal group is the most stringent criterion of graft performance.

As intimated above, the short periods of follow-up observations do not permit conclusions with respect to the ultimate fate of these grafts. Insofar as time allowed, the possible appearance of dilatation and calcification has been searched for by periodic roentgen studies, but none has as yet been observed. It may also be stressed that in the only case (operation 8) in which late thrombosis of the graft took place, the cause was not degenerative change in the graft proper but extension of the occlusive process of the adjacent distal arterial segment.

SUMMARY AND CONCLUSIONS

Beta-propiolactone in a concentration of 1 per cent by weight was found to be effective in sterilizing human arterial homografts obtained without asepsis at routine autopsies. Grafts so treated retained an essentially normal histologic structure and practically unimpaired elasticity and tensile strength.

In 31 grafting operations of varying magnitude and including 17 replacements of segments of the femoro-popliteal arterial trunk, grafts sterilized with beta-propiolactone remained patent in 90 per cent of the instances during follow-up periods of from 1½ to 13 months.

This method of sterilization has greatly increased the variety and amount

Table 2. Summary of Clinical Data

OP. SER. NO.*	CASE SER. NO.*	SEX	AGE	DATE OF OPERATION	OPERATIVE DIAGNOSIS	LENGTH OF GRAFT (CM.)	PERIOD OF FOLLOW-UP (MO.)	RESULT
					AORTO-ILIAC			
2	2	M	58	1-19-54	S.O., aorto-iliac	AoB.	10	Patent
6	5	M	46	2-2-54	S.O., rt. ext. iliac	12 cm	9½	Patent
7	6	M	60	2-6-54	Aneurysm, abd. aortic	AoB	1½	Patent†
10	9	M	53	2-22-54	S.O., aorto-iliac	AoB	9	Patent
11	10	M	58	3-20-54	S.O., lt. c. iliac	6 mm	8	Patent‡
14	11	M	64	4-13-54	Aneurysm, abd. aortic	AoB	7	Patent
17	13	M	57	5-11-54	Aneurysm, abd. aortic	AoB	6	Patent
18	14	M	45	6-3-54	S.O., aorto-iliac	AoB	5½	Patent
21	17	M	51	6-8-54	S.O., aorto-iliac	AoB	5	Patent
22	18	M	63	7-20-54	Aneurysm, abd. aortic	AoB	4	Patent
24	20	M	61	8-3-54	S.O., aorto-iliac	AoB	3½	Patent
25	21	M	42	8-5-54	S.O., aorto-iliac	AoB	3¾	Patent
27	22	M	56	9-7-54	Aneurysm, abd. aortic	AoB.	2½	Patent
30	24	F	59	9-30-54	S.O., aorto-iliac	AoB	1½	Patent
					FEMORO-POPLITEAL			
1	1	M	50	10-31-53	S.O., lt. sup. fem.	15	13	Patent
3	3	M	53	1-16-54	S.O., rt. sup. fem.	22½	10	Patent
4	4	M	57	1-19-54	S.O., rt. sup. fem.	7	10	Closed
5	3	M	53	1-28-54	S.O., lt. sup. fem.	11	10	Closed
8	7	M	66	2-13-54	S.O., lt. sup. fem.	27	7	Patent‡
9	8	M	54	2-18-54	S.O., lt. sup. fem.	30	9	Patent
12	8	M	54	3-24-54	S.O., rt. sup. fem.	24	8	Patent
13	9	M	53	4-1-54	S.O., rt. sup. fem.	32	7½	Patent
15	12	M	48	4-20-54	S.O., rt. sup. fem.	29	7	Patent
16	5	M	46	4-22-54	S.O., rt. sup. fem.	26	7	Patent
19	15	M	48	6-3-54	S.O., rt. sup. fem.	28	5½	Patent
20	16	M	61	6-5-54	S.O., rt. sup. fem.	30	5½	Closed
23	19	M	64	7-27-54	S.O., lt. sup. fem.	29	¾	Patent†
26	11	M	64	9-3-54	Aneurysm, rt. c. fem.	11	2½	Patent
28	23	M	63	9-14-54	Aneurysm, lt. pop.	20	2	Patent
29	15	M	48	9-16-54	S.O., lt. sup. fem.	40	2	Patent
31	25	M	51	10-5-54	S.O., rt. c. fem.	8	1½	Patent

Abbreviations: S.O., segmental occlusion; AoB., aortic bifurcation.
* Serial numbers of operations and cases are not identical, some patients had more than one operation for anatomically unrelated lesions.
† See text. ‡ Thrombosis distal to the graft.

ethanol, and thioglycollic acid) have been employed in processing segments of dog and pig aortas.

After unsterile arterial segments have been subjected to the agents in question, they are then stored in the same agent, in its vapors, or in absolute ethanol. Storage temperatures have been either —20° C. or ~25° C. Certain segments have been transplanted into animals, while the remainder have been subjected to tissue culture, bacteriologic, histologic, and microanalytic studies. Over 90 grafts have been implanted, mostly homografts, but including a few heterografts. Most of the grafts have been placed in the thoracic aortas of growing pigs, but a few have been inserted into the abdominal aortas of adult dogs. At sacrifice the grafts were studied grossly

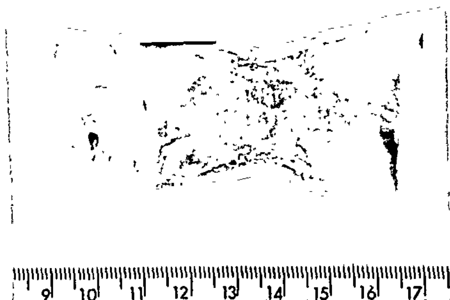


Fig 1 Ethylene imine-treated aortic homograft implanted into the thoracic aorta of a growing animal (pig No. 239) The marks of the previously used "shunt ligatures" are seen about halfway between the ends of the graft and the edges of the picture. Calcification is present in the graft.

and histologically. Growth measurements and photographs were taken and microanalytic studies were made.

RESULTS

There were seven spontaneous deaths of the recipient animals, only four of which were related to graft complications. Thirty-one animals with grafts treated with ethylene oxide, ethylene carbonate, ethylene imine, propylene oxide, and absolute ethanol have been sacrificed and form the basis for this report These animals were in apparent good health at slaughter and the grafts were patent and apparently functionally adequate. There has been no evident neoplasm or associated infection. Reaction of the tissues adjacent to the graft has been minimal, except in the case of prolonged treatment with ethylene imine.

Grafts preserved in ethylene imine presented a characteristic picture when harvested (Fig. 1). They were thin and prominently calcified. This latter change involved the adjacent aorta with extensive medial calcifica-

of vascular graft material obtainable at autopsy and has made the procurement of such grafts much simpler.

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CHEMICAL PRESERVATION OF VASCULAR GRAFTS: EFFECTS ON GRAFT COMPLICATIONS*

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While the use of vascular grafts is widely accepted, their procurement remains a problem The number of suitable donors is limited if the grafts are to be obtained in a sterile manner, and the processes of preservation are frequently complicated The sterilization of contaminated grafts is another recent approach to the problem[1,3] Some criticism of the sterilization procedures has been voiced.[4] Chemical processing of arterial segments is another method[3]

This paper is a preliminary report of our experiences with chemical processing and storage (without freeze-drying) of arterial segments and of our results after implantation of these grafts in experimental animals.

METHOD

Eight chemical agents (ethylene oxide, ethylene carbonate, ethylene imine, propylene oxide, trimethyl phosphate, beta-propiolactone, absolute

* From the Department of Surgery, University of Washington School of Medicine, Seattle This work was supported in part by Grant-in-Aid H-1136, National Institutes of Health. The Life Insurance Medical Research Fund (Grant G 54-40) supported the chemical work.

Table 1. Measurement Changes Following Implantation of 17 Chemically Preserved Grafts into Weanling Pigs

			ANIMAL		LINEAR GROWTH, PER CENT		DIAMETER GROWTH, PER CENT	
TYPE OF PRESERVATION	TYPE OF GRAFT	NUMBER OF PIGS	PER CENT WGT. GAIN	PER CENT LINEAR GROWTH	AORTA	GRAFT	AORTA	GRAFT
Ethylene oxide	Homograft	6	778	82	82	17	81	5
" "	Heterograft	2	570	74	73	50	64	43
" carbonate	Homograft	1		0	20	6	15	22
" imine	"	6	377	50	22	−1	22	18
Propylene oxide	"	1	380	85	70	24	39	−11
Absolute ethanol	"	1	470	100	150	30	90	10

tion Subintimal plaques were present in the graft. While longitudinal and diameter "growth" was restricted (Table 1) as compared to the host aorta there was more relative increase in diameter in the ethylene imine-treated grafts than following the use of the other chemical agents. It should be noted that all of the harvested ethylene imine grafts appear to have been treated excessively with the agent and none of our animals with less rigorous ethylene imine treatment has yet been sacrificed.

Ethylene oxide and ethylene carbonate-treated homografts (Fig. 2) resembled each other grossly (see also Table 1). They were thin, leathery, and slightly pliable. Gross degenerative changes were present but were less severe than we have reported as occurring in homografts otherwise preserved.[5] "Growth" was markedly reduced, especially in diameter measure-

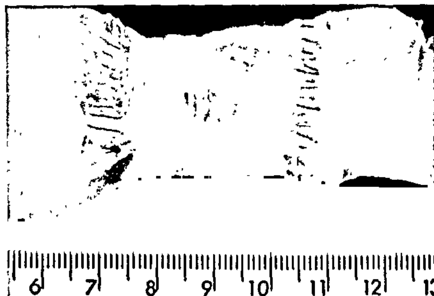


FIG 2 Ethylene oxide-treated aortic homograft implanted into the thoracic aorta of a growing animal (pig No 203) Subintimal plaques are visible but otherwise the intima appears to be in good condition.

ments, and that which did occur apparently resulted from a stretching of the graft. One ethylene oxide graft ruptured five months after implantation.

The few homografts treated with other agents and sacrificed to date (see Table 1) showed restricted "growth."

The two heterografts harvested to date (dog aorta implanted in weanling pigs) were treated with ethylene oxide. One of these (Fig. 3) was almost comparable to the host aorta at slaughter in respect to growth changes, gross appearance at slaughter, and pliability.

Prior to implantation the treated grafts showed considerable variation. Grossly they ranged from thin, leathery, and tough to thick, pliable, and soft. Reconstitution for 45 minutes in saline improved the appearance of these grafts. Prior to implantation the microscopic appearances also varied. Cellular changes were evident, especially with the ethylene imine-preserved homografts. Post-implantation sections also confirmed the gross findings.

tained less nitrogen than the normal (although not enough less to be experimentally decisive without further work), while the ethylene imine-treated graft had significantly more nitrogen than the normal tissue.

DISCUSSION

Factors to be considered in evaluating the chemical preservation of arterial grafts are (1) the facility of processing the tissue and the amount of treatment required for optimum preservation and sterilization; and (2) the alterations occurring in the physical, chemical and biologic potentialities of the tissue. Our preliminary studies indicate that the difficulties in chemically processing aortic segments are not appreciable. The influence of factors such as temperature, time of treatment, and concentration of agents will be clearer when all the grafts are harvested. Trafas and associates[3] studied these factors for beta-propiolactone.

Chemical processing alters the physical qualities of the grafts and perhaps the chemical nature of the tissue proteins as well. The degenerative changes usually present (calcification and subintimal plaques) were not as marked in some of the ethylene oxide and ethylene carbonate grafts harvested to date. Trafas and associates[3] noted slight calcification in beta-propiolactone-treated homografts, but his implants were into the abdominal aorta of adult dogs rather than into the thoracic aorta of growing pigs. We have previously noted that the former circumstance produces less degenerative change than the latter.[5]

SUMMARY

Eight chemical agents have been employed experimentally in the sterilization and preservation of aortic homografts and heterografts. Certain of these agents have advantages over others, but in the present state of our observations, it is not possible to state definitively which of them is superior from a practical standpoint. On the whole, chemical preservation of vascular grafts as used in our experiments produced reasonably satisfactory but far from perfect grafts.

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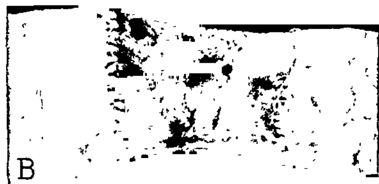
Medial calcification was often present and occasional subintimal plaques were noted

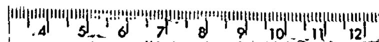
The intima was of variable thickness, but was usually intact. The general normal architectural pattern remained, but hyaline deposits were extensive There was, of course, no microscopic evidence of actual survival of the cellular components of the graft

Microanalytic Studies. Preliminary evidence appears to indicate that









several days. In respect to growth changes and gross appearance this graft was surprisingly like the host aorta

both ethylene oxide and ethylene imine, as used in these experiments, reacts with the non-lipid portion of the tissue At the same time a large portion of

now sutured into the defect with continuous everting mattress sutures of 5-0 arterial silk. The distal clamp was released for approximately 3 minutes to allow sealing of the suture lines. The proximal clamp was then slowly released, and not reapplied after reestablishing the arterial flow. Time of occlusion varied from 20 to 32 minutes.

In addition, fresh, non-lyophilized thoracic aorta grafts of one cat and two rabbits were investigated, using the same operative technique.

RESULTS

Fresh Grafts (Table 1). Three fresh grafts (2 rabbit and 1 cat) were implanted into puppies for 7 days, 17 days, and 12 months, respectively. The

Table 1. Results of Fresh Non-lyophilized Heterografts

SOURCE	PERIOD OF IMPLANTATION	LENGTH	APPEARANCE AFTER IMPLANT	LOCATION OF IMPLANT	FATE OF GRAFT
1 CAT T AORTA	17 DAYS	2.5 CM.		T AORTA	DEATH—PNEUMONIA PATENT GRAFT WITH 2-3 MURAL THROMBI
2 RABBIT T. AORTA	12 MONTHS	3.0 CM		T. AORTA	NO ANEURYSM GRAFT PATENT 8—10 MONTHS BY AORTAGRAM AND AT POSTMORTEM
3 RABBIT T. AORTA	7 DAYS	2.5 CM		T. AORTA	DEATH—HIND-QUARTER PARALYSIS GRAFT PATENT, NO ANEURYSM OR FRACTIONATION.

7 and 17 day old grafts showed marked foreign-body reaction with adhesions to lung, diaphragm, or both, on death of the animal. Visualization of the third fresh graft by open thoracotomy after it had been in place for eight months revealed marked adhesions of lung to graft. There was a narrowing of the aortic lumen for the length of the graft, with a palpable thrill distally. The graft demonstrated little or no relative increase in its diameter, even though the dog had quadrupled in size.

Lyophilized Grafts (Table 2). Seven lyophilized rabbit aorta grafts were

Table 2. Results of Lyophilized Heterografts

SOURCE	PERIOD OF IMPLANTATION	LENGTH	APPEARANCE AFTER IMPLANT	LOCATION OF IMPLANT	FATE OF GRAFT
1. RABBIT T AORTA	4 DAYS	2.5 CM.		T. AORTA	TRANSVERSE FRACTIONATION (DEATH—DISTEMPER)
2 RABBIT T AORTA	21 DAYS	2.5 CM		T AORTA	FUSIFORM ANEURYSM RUPTURE 21 ST DAY
3. RABBIT T. AORTA	6 DAYS	2.5 CM		T. AORTA	TRANSVERSE FRACTIONATION NO FOREIGN-BODY REACTION (DEATH—DISTEMPER)
4 RABBIT T AORTA	12 MONTHS	2.5 CM.		T AORTA	FUNCTIONED WITH FUSIFORM ANEURYSM FOR 9 MONTHS (SACRIFICED)
5. RABBIT T AORTA	6 WEEKS	2.5 CM		T. AORTA	TRANSVERSE FRACTIONATION, SUBINTIMAL HEMORRHAGE, NO ANEURYSM. (DEATH-DISTEMP)
6 RABBIT T. AORTA	7 WEEKS	2.5 CM		T AORTA	PATENT GRAFT, SACCULAR ANEURYSM (SACRIFICED)
7 RABBIT T. AORTA	10 MONTHS	2.5 CM		T AORTA	FUSIFORM ANEURYSM AT 6 WEEKS BY AORTAGRAM (SACRIFICED)

THE DEVELOPMENT OF EXPERIMENTAL ANEURYSMS IN LYOPHILIZED ARTERIAL HETEROGRAFTS*

LEON J. TUNE AND J. CUTHBERT OWENS

Since the advent of modern surgery, clinicians interested in vascular research have been desirous of some method of producing aneurysms in experimental animals. When the subject of aneurysms and their treatment by graft replacement occupies such prominence in the pages of present-day surgery, a method of producing arterial aneurysms experimentally with demonstrable stages in their development would seem appropriate.

We present a small series of separate fresh or lyophilized thoracic aorta segments from cat and rabbit, transplanted into the thoracic aorta of mongrel puppies and observed for four days to twelve months. The fresh grafts showed principally foreign-body reaction and mural thrombi. However, all lyophilized rabbit grafts revealed transverse fractionation of the media or aneurysmal formation in four days to seven weeks.

Deaths from distemper, operative shock, and hind-quarter paralysis in these small dogs made it difficult to accumulate a larger number of dogs for a desirable postoperative period of observation.

TECHNIQUE

White rabbits and mixed-breed cats weighing nine to ten pounds were sacrificed with ether The animal's chest wall was surgically prepared by shaving and cleansing of the skin with 1:1000 tincture of Zephiran The operative area was draped The entire thoracic aorta was excised through the seventh intercostal space, using aseptic technique. This aorta was divided into 2 5 cm. segments. The loose adventitia was removed by sharp dissection. These arterial segments were placed separately into special glass tubes and quick-frozen in dry ice and 95 per cent isopropyl alcohol at minus 76° C. The tube was packed in dry ice, and suction was applied by means of a lyophilizing machine which reduced the internal pressure to approximately one micron of mercury pressure Suction was continued for approximately 24 hours, when the vessels were considered to be completely dried. The tubes were heat-sealed, and the grafts stored in the vacuum tube at room temperature from one to thirty days

Thirty minutes before using the grafts, the glass tubes were filled with Ringer's solution, restoring the graft to its original appearance.

These grafts were now readied for placement into the recipient. Twelve to fourteen pound mongrel dogs were anesthetized with intravenous sodium pentobarbital, and an endotracheal tube was inserted and connected to an automatic respirator The left chest and abdomen was prepared with 1.1000 tincture of Zephiran solution and draped, the left chest was entered through the ninth intercostal space The lower thoracic aorta was mobilized, four intercostal arteries were ligated and transected, and the loose adventitia stripped from the aorta A 2 cm. segment of aorta was excised following the proximal and distal application of Potts aortic clamps. The heterograft was

of the fusiform aneurysms ruptured at 21 days (Fig. 2). Marked edema was observed in the aneurysmal wall, with stenosis still present at each suture line. No organ was adherent to this graft. Aortograms of the remaining two grafts implanted 5 weeks to 3 months demonstrated fusiform aneurysms (Fig. 3). Gross observations of the lyophilized grafts as contrasted to the fresh grafts demonstrated a significant reduction of the foreign-body reaction in the former.



Fig 3. Aortograms, 5 weeks (A) and 3 months (B), respectively, after lyophilized rabbit aorta grafts implanted into thoracic aorta of dogs Note fusiform aneurysms.

Microscopically, by three weeks these lyophilized heterografts showed early extensive replacement by cellular granulation tissue, with the largest amount adjacent to the host aorta. However, there was a minimal amount of foreign-body reaction. Distal to the anastomosis, the graft became progressively thinner and was attenuated to less than one-third the width of the host aorta in some areas. At these points of thinning, the elastic fibers of the media and internal elastic membrane were completely absent (Fig. 4). In this early period, the internal elastic membrane was fairly well intact adjacent to the anastomosis, having the usual convoluted appearance, but progressively losing these folds and diminishing until there was a point of interruption or complete absence at the thin portion of the graft (Fig. 5). A 2-week-old graft ruptured at one of these areas. Within 12 months, the heterograft was almost completely replaced by dense fibrous connective tissue. The foreign-body reaction was present, but not marked. All elastic fibers were absent except in one area of the 12-month-old graft where there appeared to be numerous small blood vascular spaces and capillaries in the adventitia and media immediately adjacent to the surviving elastic fibers. Arterioles and a small artery were noted in the surrounding loose areolar tissue at this point (Fig 6).

implanted into puppies for 4 days to 12 months. Two grafts in place 4 to 6 days showed transverse fractionation of the media on autopsy (Fig. 1). One graft implanted for 6 weeks showed transverse fractionation with subintimal hemorrhage (Fig. 1). Three grafts implanted 3 weeks to 12 months showed fusiform aneurysms, one graft presented a saccular aneurysm. One



Fig 1 Lyophilized rabbit aorta grafts implanted into thoracic aorta of dogs for 6 days (*A*) and 6 weeks (*B*), respectively Note transverse fractionation (*A,B*) and subintimal hemorrhage (*B*)

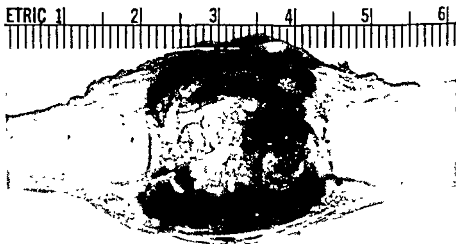


Fig 2 Lyophilized rabbit aortograft implanted into thoracic aorta of dog, demonstrating ruptured fusiform aneurysm at 21 days. Note stenosis distal to proximal suture line, which represents caliber of graft at time of implant.

ically to be similar reproductions of
aorta. Fractionation of the media, s
tion, and eventual perforation at a p
brane defect would seem comparable.

As previously described by Hufnagel,[1] the marked reduction of the foreign-body reaction was observed only in the lyophilized heterograft. This foreign-body reaction as found in the fresh heterografts tended to strengthen the graft conduit by its sclerosing effect and rapid fibrosis. However, this reaction appears to invite mural thrombi and complete thrombosis of the graft.

In considering the possible reasons for the formation of the aneurysms in these grafts, we might query them as being the result of the following factors:

1. *Post-stenotic Dilatation Effect.* The caliber of these grafts was approximately 50 to 60 per cent smaller than the recipient host aorta. This stenosis extended the length of the graft, and one would expect the post-stenotic dilatation to occur in the host aorta just distal to the graft.[2,3] This is in accordance with the Venturi and the Bernoulli theorem, basically meaning that the lateral pressure is inversely proportional to the velocity of flow; such did not occur, but rather the dilatation was confined to the graft.

2. *Stress Limit and Structural Fatigue.* The rabbit's aorta wall is approximately one-quarter as thick as the dog's aorta wall. Therefore, one surmises that its stress limit might be considerably less If this be true, and structural fatigue of the graft by stretching occurs before, during, or after implantation, ballooning of the graft would develop This effect plus stenosis of the proximal suture line sets an ideal stage for aneurysmal dilatation.

3. *Reduction of Stress Limit Produced by Lyophilization.* If we adopt the accepted belief that the lyophilized graft is non-viable, then we would be justified in assuming that the stress limit of a non-viable graft is reduced from that of its viable state, and if subjected to repeated pulsations, structural fatigue supervenes. Subsequent fractionation of its media and internal elastic membrane occurs, leading to rupture of the graft or the birth of an aneurysm.

SUMMARY AND CONCLUSIONS

1. A small series of 3 fresh and 7 lyophilized thoracic aorta grafts from cat and rabbit were transplanted into the thoracic aorta of mongrel puppies and observed for 4 days to 12 months.

2 The fresh grafts showed principally foreign-body reaction and mural thrombi However, all seven of the lyophilized rabbit grafts revealed transverse fractionation of the media or aneurysmal formation in 4 days to 7 weeks.

3. Microscopic examination of the lyophilized heterograft at 3 weeks showed extensive replacement by cellular granulation tissue, minimal foreign-body reaction, and diminution to complete absence of the media elastic fibers and internal elastic membrane. This degeneration continued until there was nearly complete replacement by dense fibrous connective tissue at 12 months. A few elastic fibers remained adjacent to a vascularized area.

4. Physical factors including post-stenotic dilatation, stress limit, structural fatigue, and their relationship to the gross and microscopic findings in

DISCUSSION

Experimental aneurysms produced by implantation of lyophilized rabbit aortic grafts into the thoracic aorta of dogs appear grossly and microscop-



Fig. 4 Fig 5

Fig 4 Photomicrograph of lyophilized rabbit aorta graft implanted into thoracic aorta of dog 21 days. Intimal side up Note cellular granulation tissue replacement, absence of media elastic fibers and intimal elastic membrane Section taken adjacent to point of rupture Weigert elastic stain, ×100

Fig 5 Photomicrograph of lyophilized rabbit aorta heterograft implanted into thoracic aorta of dog 21 days. Intimal side up Note convolutions of intimal elastic membrane progressively flattening and thinning Section taken near anastomosis Weigert elastic stain, ×100



[illegible]

The most important criteria for the evaluation on an arterial graft over a given period of time in the experimental animal are the gross appearance of the graft and the condition of the animal. However, it is necessary clinically to select the type of graft which is expected to give the best results over a period much longer than experimental animals have been followed at the present time. It appears likely that the best basis for making this decision is the relative condition of the elastic fibers in the types of grafts being considered.

Experiments have demonstrated that the elastic fibers of freeze-dried arterial homografts in dogs are in as good or in better condition after periods of observation up to one year than those of comparable fresh homografts.[1] It has now been possible to chemically damage the elastic fibers of these grafts, without producing other demonstrable changes in the tissue, and a comparison of the performance of the treated and untreated grafts is of interest

Experiments were performed to develop a technique for the sterilization of arterial grafts taken at non-sterile autopsy using a small quantity of ethylene oxide[4,5] vapor in a closed system. This method was used to avoid the explosion hazard of large quantities of vapor being released into the room from the use of the ethylene oxide liquid.[6] Forty-five minutes to one hour treatment by the vapor was found, in our experiments, to be the minimum sterilizing dose for vessels moderately heavily contaminated with standard pathogenic organisms. To obtain information regarding the margin of safety between the minimum sterilizing dose and the maximum safe dose for the grafts, vessels were treated for 20 hours.

METHOD

Aorta was removed at ordinary postmortem examination on dogs and inserted in small test tubes. These tubes were placed in a desiccator jar and approximately nine-tenths of the air removed. The jar was then connected to a supply of liquid ethylene oxide maintained at 0° C., ten degrees below its boiling point. After 20 hours the vessels were removed and frozen by lowering the tubes in a mixture of dry ice and alcohol. They were stored at dry ice temperature until dried directly from the frozen state in a vacuum of 50 microns of mercury for 24 hours The grafts were then sealed under vacuum in small ampules, and preserved at room temperature for several weeks. The vessels were reconstituted by immersion for one hour in normal saline, and implanted in the abdominal aorta of dogs using aseptic technique

RESULTS

Grafts were implanted in ten dogs. One dog died on the first postoperative day from rupture of the graft, and an occlusive thrombus developed in another. One of the grafts appeared essentially normal at sacrifice three months later, but the remaining seven dogs developed aneurysms which were two to five times the original diameter of the graft. One of these aneurysms ruptured spontaneously one month after implantation, and the others were discovered at sacrifice three months later.

The aneurysms were fusiform in all of the dogs. In some, they were symmetrical while in others they originated largely from one side of the graft, usually that lying ventral in the recipient dog In two the media had

the lyophilized rabbit aorta heterograft, have been briefly discussed. These factors emphasize certain fundamentals in vascular grafting, namely, that any arterial graft should closely approach the caliber and wall thickness of the recipient host aorta, with no significant stenosis of the suture lines.

5 In this small series of animals, it appears that the lyophilized rabbit aorta graft implanted into the thoracic aorta of the dog consistently produced transverse fractionation of the elastic fibers and resultant aneurysmal formation.

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THE IMPORTANCE OF ELASTIC LAMELLAE IN AORTIC GRAFTS, AND A TECHNIQUE FOR THE EXPERIMENTAL PRODUCTION OF AORTIC ANEURYSMS*

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It is becoming generally agreed that the cells of a homologous arterial graft, whether viable or non-viable at the time of implantation, disappear and are replaced by fibroblasts from the host. Muscle cells degenerate rapidly and apparently are not replaced[1] The extracellular components of the graft persist for a longer period of time Some collagen fibers persist for as long as a year, but the majority are gone after several months The elastic fibers, on the other hand, tend to persist for years, with only a minor decrease in number. Although the host is able to replace collagen fibers by invading fibroblasts, there is no evidence that new elastic fibers are created.

Experiments reported in the literature, such as those of Sako,[2] have demonstrated that fibrous tubes constructed from such material as pericardium dilate after a period of exposure to arterial pressure, unless they are very heavily reinforced by fascia or other tissue. Veins which contain fewer elastic fibers than arterial homografts tend to form aneurysms if implanted in the unsupported thoracic or abdominal aorta[3]

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** The opinions or assertions contained herein are the private ones of the writers and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

were implanted in 40 dogs in a previous study. There were no aneurysms in that series, and few mural thrombi.

In this former series of grafts, the elastic fibers appeared somewhat swollen, and were decreased in number by about one-fifth but in all cases remained in large numbers in the wall of the graft. There were no areas in which the elastic fibers were completely absent. In the one dog in the presently reported series in which an aneurysm did not form, there were no complete breaks in the elastic fibers and only minor losses in any single area (Fig. 2). The four small-to-moderate aneurysms showed areas in which a majority of the elastic fibers had disappeared. The three large

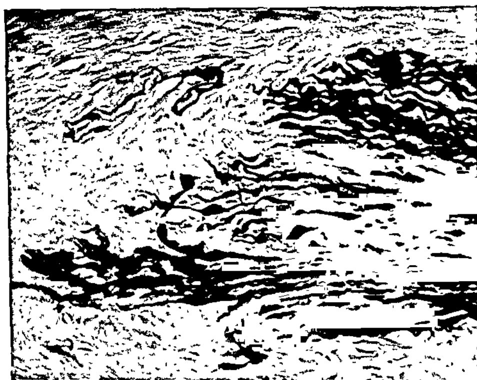


Fig 3 Media of a large aneurysm at the edge of a break in the elastic lamellae Verhoeff's stain, ×120.

aneurysms showed total loss of the elastic fibers in the major portion of the graft wall. Host fibroblasts invaded the graft wall, but apparently were not able to prevent dilatation (Fig. 3). No significant changes were noted in the appearance of the elastic fibers which remained.

The experimental [illegible]
in [illegible]
by [illegible] necrosis of the media with nitrogen mustard. These aneurysms ruptured spontaneously after two or three weeks. German[8] surgically constructed small saccular aneurysms by anastomosing a blind pouch of autogenous jugular vein to the side of the carotid artery. Overtreatment of grafts could probably be satisfactorily used for the experimental production of larger fusiform aneurysms for study over a period of several months.

SUMMARY

Freeze-dried homologous arterial grafts were drastically overtreated with

separated entirely, showing the adventitia from inside the graft. All of the aneurysms but one contained a moderate to large sized mural thrombus (Fig. 1). These findings are in contrast to those obtained when otherwise similar freeze-dried homologous arterial grafts taken at sterile postmortem

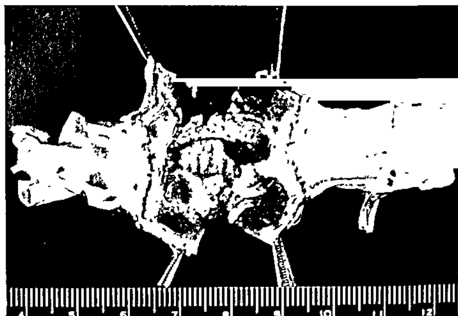


Fig 1 A large aneurysm, showing mural thrombus and splitting of the media

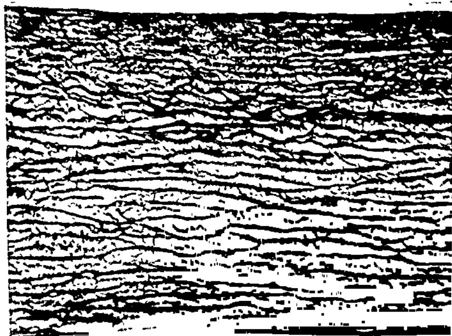


Fig. 2. The media of the one graft which did not form an aneurysm, showing essentially the same structure as undamaged freeze-dried homograft Verhoeff's stain, × 120.

demonstrated the bactericidal effect of gamma radiation. It is effective against organisms ranging in size from viruses to yeasts. The bactericidal dose for each organism depends upon *individual susceptibility, size, morphology, and physical state.* A review of these studies suggested that a dose of 2,000,000 rep (roentgen-equivalent-physical) would destroy all organisms normally encountered as contaminants of blood vessel homografts. Some of the organisms which we frequently encountered as contaminants were *Staphylococcus albus* and *aureus, Bacillus subtilis, Streptococcus faecalis,* [illegible] produced within tissue cells, it was [illegible] the changes produced by *gamma radiation would alter the structural integrity* of arteries to a degree that would preclude their use as grafts. In an attempt to answer this question, segments of dog aorta which had been exposed to gamma radiation were examined for any microscopic changes and were also given a clinical trial by being inserted as homografts in the thoracic aorta of dogs.

METHODS

Graded doses of gamma radiation ranging from 15,000 to 4,000,000 rep *were administered to fresh segments of a* dog aorta which were (1) placed in dry, sterile test tubes, (2) placed in sterile 0.9 per cent sodium chloride solution, and (3) lyophilized. The cobalt^{60} source rests in a water well fourteen feet deep, directly below a room with solid concrete walls four feet thick. The specimens to be irradiated are placed about the upper end of the well, and the source is then raised up into the room, exposing the specimens to the gamma rays. Approximately sixteen hours of exposure are *required for 4,000,000 rep of irradiation. Since gamma radiation* produces ionization, certain rather strong oxidative reactions occur in the presence of water. Because of this, it was felt that the presence of water at the time of irradiation might result in greater damage to the grafts. Therefore the effects of gamma radiation on the segments of aorta were compared when administered (1) in the presence of the water of the tissue itself, (2) in the presence of a water and an electrolyte medium, and (3) in the absence of water (lyophilization). Four groups of controls were used: (1) segments fixed in 10 per cent Formalin immediately after removal, (2) segments refrigerated at 1° C. for a time equal to that required *for irradiation,* (3) segments stored at room temperature for a similar length of time, and (4) [illegible] per cent sodium chloride solution. [illegible] Formalin and microscopic sections [illegible] with its elastic fibers is the structurally important layer of the vessel graft, Verhoeff elastic stain was used in addition to hematoxylin and eosin stains. Consistent microscopic changes attributable to gamma radiation could not be detected *in any of the* irradiated segments of aorta (Fig 1). There was some patchy fragmentation of the elastic tissue fibers, but this could not be correlated with the amount of irradiation given. Since microscopic examination did not reveal any differences between the irradiated and control segments, it alone was not considered an adequate method for evaluating the effects of gamma radiation.

Irradiated segments of dog aorta were placed *in the thoracic* aortas of

ethylene oxide and implanted in the abdominal aorta of dogs. Most of the dogs developed aneurysms which were very well correlated with areas of partial or complete destruction of the elastic fibers. These experiments demonstrate the danger of overtreatment with ethylene oxide, and also provide a reasonably reliable method for the experimental production of fusiform true aneurysms

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THE USE OF COBALT[60] AS A STERILIZING AGENT FOR AORTIC HOMOGRAFTS*

I. Effect of Gamma-ray Irradiation upon the Structural Integrity of the Graft

JACK A. MACRIS, HERBERT SLOAN, AND JOHN E. OREBAUGH**

The increasing demand for vessel grafts has made it difficult for most hospitals to maintain an adequate supply. Many otherwise suitable grafts have had to be discarded because they were contaminated either by infection existing in the donor or by improper handling during removal and preparation of the grafts for storage. A simple method of sterilizing contaminated grafts would greatly increase the number of usable grafts However, any technique of sterilization must preserve the structural integrity of the graft as well as destroy the contaminants. Ethylene oxide[1] and beta-propiolactone[2, 3] have been employed successfully to sterilize vessel grafts, as has irradiation from the Van de Graaff[4] and Capacitron machines[5]

Extensive bacteriologic studies[6, 7] carried out at the University of Michigan with a 10,000 curie source of gamma radiation from cobalt[60] have

* From the Department of Surgery, University of Michigan, Ann Arbor This work was supported in part by funds from the Michigan Heart Association

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anticoagulants were employed at any time. 400,000 units of penicillin (S-R) were administered intramuscularly daily for five days postoperatively.

RESULTS

Cultures were made following irradiation of seven contaminated grafts stored in Tyrode solution, and no organisms were recovered. Thirty-one cultures were made of the reconstituting solution (0.9 per cent sodium chloride) of lyophilized, contaminated grafts which had been irradiated. One culture contained a few gram-negative anaerobic bacilli. Because of the very small number of organisms found and because the reconstituting solution was exposed to the air for a period of at least one hour, this single positive culture may well have been due to contamination at the time of reconstitution rather than to survival of organisms following irradiation.

Four of the sixteen dogs operated upon died within three days from complications which included renal and spinal cord damage, operative hemorrhage, and atelectasis. Gross examination of the grafts in these animals revealed no changes except for the formation of a wedge-shaped thrombus at the suture lines after the second day. Microscopically, there was loss of the intima of the graft on the first day, and also patchy loss of staining reaction of the nuclei of the lyophilized grafts. It is interesting to note that this patchy loss of nuclear staining, which was interpreted as evidence of cellular necrosis, was not evident at the time of reconstitution of the lyophilized grafts, but occurred only after implantation of the grafts. This probably indicates that even though the cells do not survive the process of lyophilization, they are so perfectly preserved morphologically as to undergo the same staining reactions as do living cells.

Seven of the surviving twelve dogs are alive and well, after periods ranging from one to four months since their operation. All of these dogs have excellent pulses in the hind legs. Five dogs were sacrificed at intervals ranging from three weeks to forty-five weeks following insertion of the graft. At three weeks the graft showed a red, finely granular intimal surface; about one centimeter of the length of the graft had been covered with endothelium. There were no thrombi and the graft was patent. The second dog was sacrificed at the end of eight and one-half weeks Externally there was no disparity in size between the host aorta and the graft, and the adventitial surface was smooth and continuous with the host vessel. The intimal surface was gray and glistening, and in some areas was finely wrinkled. Microscopically, the graft was completely covered by intima. In the third dog, sacrificed at the end of nine weeks, the graft was patent but the intimal surface displayed two red, shaggy areas of erosion measuring four and six millimeters in diameter. There was no thrombosis or calcification at these sites and the remainder of the intimal surface was smooth and glistening. The fourth dog was sacrificed at the end of twenty and one-half weeks (Fig 2). There was a slight narrowing at the suture lines which was attributed to the small size of the graft originally. The intimal surface was smooth, glistening, and in no way different from the host in gross appearance. The fifth dog was sacrificed at the end of forty-five weeks. Externally, the contour of the graft matched that of the host vessel perfectly. The intimal surface was smooth and glistening. In one portion of the graft there were two pale yellow areas measuring three millimeters in diameter which

sixteen dogs. The grafts had been obtained without aseptic precautions within one hour after death of the donor dog. Cultures from the segments of aorta showed *Streptococcus viridans*, *Staphylococcus aureus*, paracolon bacilli of the aerogenes type, and numerous gram-negative bacilli. The segments of aorta were preserved in Tyrode solution or were lyophilized and







[illegible] *(b)* seg- [illegible]erhoeff

then exposed to 2,000,000 rep The irradiated segments were stored in Tyrode solution for periods ranging from seven to nineteen days The lyophilized segments were stored from thirteen days to seven months. Culture specimens were taken from the storage solution or reconstituting solution at the time of insertion of the aortic grafts. Blood flow to the distal aorta was maintained with a polyethylene shunt while the grafts were sutured in place with continuous everting mattress sutures of 5-0 silk. No

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could not be identified microscopically. Grossly, there was no evidence of sclerosis or degenerative change in any of the grafts. Microscopically, the changes observed were loss of intima on the first day of implantation, loss of nuclear st
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the graft or in fibroplastic proliferation around the adventitial surface of the graft The elastic fibers in the grafts remained intact and appeared unchanged. Mucoid degeneration in isolated areas was noted in several of the

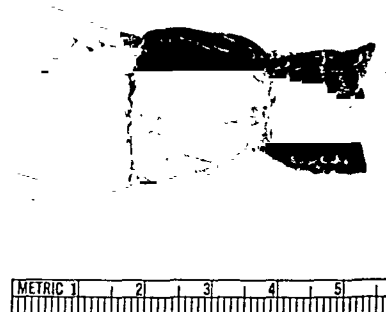


Fig 2 Photograph of an irradiated, lyophilized aortic homograft 145 days after insertion

grafts beginning as early as two months after insertion. The ten-month-old graft showed one small area of calcification near the distal anastomosis

From these results, it was felt that no adverse changes in the aortic segments could be attributed to gamma radiation and that the irradiated aortic segments functioned well as homografts when inserted into the thoracic aorta of dogs. Further evaluation of the method is of course necessary and should include a longer period of observation of the implanted grafts. Tensile strength studies of irradiated grafts are in progress. Also, more extensive bacteriologic studies with regard to viruses are under way and may indicate the necessity for a higher dose of irradiation.

CONCLUSION

Preliminary studies suggest that gamma radiation from a cobalt^{60} source does not destroy the structural integrity of aortic homografts in dogs, and that such radiation provides a simple and effective method of sterilization

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ESOPHAGUS, STOMACH, AND INTESTINE

INTRODUCTION

WARREN H. COLE

Esophagitis. The recent work of Allison, Barrett, Wangensteen, Sweet and others has done much to clarify the etiology of esophagitis, but the relationship between esophagitis, hiatus hernia and benign stenosis of the terminal esophagus is still not clear except that reflux of highly acid gastric juice into the esophagus appears to be the most important factor in production of esophagitis.

Previous investigators have found that in dogs subjected to 50 per cent proximal gastrectomy followed by esophagogastrostomy, over half die of spontaneous esophageal complications in an average of 56 days.

Papers in this year's Forum emphasize the mechanical role in pathogenesis of esophagitis, i.e., the importance of a cardiac sphincter, and report on efforts to construct a valvular mechanism if it is lost. Dillard et al., of the [illegible] vent this esophagitis in animals [illegible] junction, by construction of a [illegible], at the point of its anastomosis with the stomach, with the hope this valve would prevent reflux and its consequent esophagitis. They report 11 of 20 valves so constructed were competent, and that the valve tends to protect against the ulcer-producing effects of histamine.

Watkins and associates, of the University of Colorado, likewise have performed experiments constructing a valve equivalent to a hiatal valve in the esophageal portion of the esophagogastric anastomosis. Dogs having this valve constructed after resection of the cardia have survived 281 to 452 days without esophagitis and its complications.

Giuseffi and associates, from the Mayo Foundation, conducted experiments on dogs to determine whether or not excision of the valve-like mechanism at the cardia (in the form of the left crus) would result in esophagitis. Esophagoscopic examination performed at intervals after excision of this structure has shown that esophageal ulcerations develop in most of these dogs 8 to 10 weeks after operation.

Accordingly, overwhelming evidence is accumulating to show that the valve (formed by the left crus of the diaphragm) at the gastro-esophageal junction is a very important structure and that in its absence reflux and esophagitis will occur. Since the development of most hiatus hernias would destroy the valve-like mechanism created by the left crus, it is now understandable why stenosis and esophageal perforation occur so often in hiatus hernias.

Surgical Physiology of the Stomach. You will recall the recent work of Dragstedt and associates revealing the role of the antrum in acid production by the stomach. On the basis of their work Dragstedt and associates conclude that duodenal ulcers are due to a hypersecretion of gastric juice of nervous origin and that gastric ulcer is caused by a hypersecretion of gastric juice due to antrum hyperfunction. This may be an oversimplification

Total Gastrectomy. In an effort to shed light on the etiology of malnutrition following total gastrectomy, Oppenheimer et al., of the University of California, have performed some experiments in dogs to try to find out if intestinal absorption is decreased after total gastrectomy. After studying carbohydrate, nitrogen and fat metabolism following this operation, they concluded that the rate of absorption was actually increased, although the passage of food through the intestinal tract was more rapid. They stressed the value of not by-passing the duodenum—a point revealed by Everson's experiments a year or two ago.

In order to overcome the deficiency of intestinal absorption following total gastrectomy, Briggs et al., of the University of California at Los [illegible] gastric res- [illegible] performed. [illegible] duplicated segment of jejunum improves the absorption of food and thereby minimizes the malnutrition so commonly observed following total gastrectomy.

Antibiotics in Intestinal Strangulation. Cohn, of Louisiana State, conducted animal experiments on the role of antibiotics in the prevention of gangrene of the intestine and death following intestinal strangulation. His results indicate that preoperative bowel sterilization is not essential, insofar as antibiotic therapy following experimental strangulation obstruction prevented gangrene and indefinitely prolonged survival in experimental animals. His experiments are very striking (though difficult to assimilate) and indicate that more attention should be given to antibiotic therapy in intestinal obstruction.

of the problem and is no doubt controversal, but nevertheless it is an attractive theory

Botta et al of the Mayo Clinic reported on some experiments at this Forum session which have confirmed Dragstedt's experiments. They found an increase in volume and acidity of gastric juice collection from Heidenhain pouches, after the antrum was transplanted to the colon. This confirms the clinical impression held for the past few years that when a gastrectomy is performed for a duodenal ulcer, the antrum must be removed.

Gentry et al. of Yale conducted some experiments to determine the mechanism of action of ACTH on the antrum They measured the volume and amount of acid in gastric secretion in animals with and without the vagi intact. They found that with the antrum in place but the vagi cut, ACTH produced copious secretion with high acid. Therefore, they concluded that the stimulating effect on the antrum by ACTH does not take place by way of the vagi.

Two different groups of investigators have conducted some experiments transplanting the stomach and jejunum into different positions in the gastrointestinal tract to see what effect this change in position would have on ulcer formation. Kelly and Wangensteen, of Minnesota, sectioned the jejunum 60 cm. distal to the ligament of Treitz and transplanted the stomach into that position, leaving the vagus nerves intact Intestinal continuity was restored by an esophagoduodenostomy. Half the animals died of perforated ulcer in the jejunum just distal to the pyloric-jejunal anastomosis. They concluded that duodenal secretion going into the stomach stimulated gastric production with hypersecretion of gastric juice, or that the increased susceptibility of the jejunum was created by the transplantation. Dillard and Merendino, of the University of Washington, report on another type of interposition of intestinal organs in relation to formation of peptic ulcer. They cut across the gastro-esophageal junction and interposed a segment of jejunum between the esophagus and stomach The animals were then subjected to histamine stimulation. Results indicated the interposed jejunal segment was more resistant to acid-peptic digestion than either the stomach or the duodenum. However, other reports indicate the jejunum is less resistant. A major difference is that in these experiments gastric secretion does not flow over the jejunum. The interposed segment acted as a physiologic sphincter and prevented proximal esophagitis.

The argument as to how much stomach should be resected for duodenal ulcer still continues. It is agreed that a high resection is not required for gastric ulcer, but that two-thirds to three-quarters of the stomach should be resected for duodenal ulcers There is likewise controversy as to whether an anterior or posterior anastomosis is desirable from the standpoint of minimizing the incidence of postoperative gastrojejunal ulcer. State et al., of the University of Southern California, have conducted some experiments showing that at least 50 per cent of the stomach must be resected in dogs to protect against histamine induced ulcer. They noted further that if the antrum was not resected with the gastric resection the incidence of peptic ulcer was lessened. This seems paradoxical to clinical experience because we are learning that gastrectomy without removal of the antrum usually fails to relieve the ulcer diathesis. The inconsistency is apparently explained by the fact that these authors are reporting on the protective influence of the antrum against the ulcer produced by histamine.

seromuscular approximation. The determination of extent of gastric resection was aided by preliminary trials on sacrificed animals. In all experiments the weight of the resected portion was compared with that of the residual pouch, at the time of death or sacrifice, to determine the percentage of resection.

The animals were allowed to recover from the initial surgery for approximately one month and then placed on daily injections of 30 mg. of histamine base in beeswax, given intramuscularly as described by Hay et al.[3] These injections were given for a period of 30 days unless the animal died before that period was up. In group III dogs at re-operation the stomach was opened widely and no ulcers found. The antrum was excised and a Billroth I or II procedure performed. They were allowed a recovery period of 30 days and then again placed on histamine in beeswax for an additional 30 days.

OBSERVATIONS

Control Group I Of 11 dogs, 7 developed ulcers.

Group II. A. Of 16 dogs with "sleeve" resections, 2 developed ulcers.

B. In 15 dogs with a "wedge" resection, one developed an ulcer.

Thus in a total of 31 dogs, 3 developed ulcers (10 per cent).

Group III. In this group, where the antrum was resected in a second operation, of 3 dogs with "sleeve" resection, two developed ulcers, and of 3 dogs with "pie" or "wedge" resection, all developed ulcers. Thus of a total of 6 dogs, 5 developed ulcers.

Table 1. Incidence of Histamine Induced Peptic Ulceration

GROUP	NO OF DOGS	NO DEVELOPING ULCERS
Group I	11	7
Group II A	16	2
Group II B	15	1
Group III	6	5

DISCUSSION

These results would seem to indicate that following a 50 per cent resection of the acid secreting portion of the stomach, the incidence of ulceration, following the injection of histamine in beeswax, is greater in those animals in whom gastro-intestinal continuity has been restored by the Billroth I or II technique than in those in whom continuity has been restored by anastomosing the residual gastric pouch to the antrum. The high incidence of ulceration following resection of the antrum and anastomosing the unchanged gastric pouch to the duodenum or first portion of the jejunum also favors the concept that when the antrum is not separated from the residual pouch it tends to protect the animal from histamine induced ulceration.

These observations confirm the findings of Kelly and co-workers,[5] who noted only one ulcer in 12 dogs with tubular resection of approximately 40 per cent of the acid secreting area of the stomach.

The different effect on gastric secretion exerted by the antrum when it is excluded and when it is in continuity is not clearly understood. Ivy and Oyawa[4] showed that the secretions of the pyloric antrum do not contain any acid. According to Babkin,[1] Sokolow showed that 0.5 per cent HCl introduced into the stomach markedly diminished the secretion of gastric

THE ROLE OF THE PYLORIC ANTRUM IN EXPERIMENTALLY INDUCED PEPTIC ULCERATION IN DOGS*

DAVID STATE, ALFRED KATZ, ROBERT S. KAPLAN, BERNARD HERMÁN, LEON MORGENSTERN, AND IRVING A. KNIGHT

Although a "three-quarter" gastric resection is most widely practiced for medically resistant duodenal ulcer, still it is not an ideal operation for there are a number of difficulties attending its use Amongst these are closure of a badly scarred duodenum, dumping syndrome and failure to gain weight. In an effort to overcome these problems, Wangensteen and co-workers[6] have revived and modified the segmental gastric resection, taking at least 85 per cent of the acid secreting portion of the stomach and anastomosing the residual gastric pouch to the antrum. There has been concern, on most surgeons' part, about leaving the antrum, because of the poor results attending the antral exclusion operation. It is accepted that gastrin, which is a strong gastric acid secretagogue, is elaborated here. Clinical experience has shown repeatedly that, in the Eiselberg antral exclusion operation, the incidence of stomal ulcer is high and the mere removal of the antral mucosa will result in the healing of a stomal ulcer. When, however, the antrum is not excluded, its influence on gastric acid secretion may be different. The experiments herein reported were directed at elucidating the function of the antrum when left in continuity with a residual segment of gastric pouch.

METHOD

Three groups were operated upon as follows:

Group I. A control group of 11 dogs in whom a 50 per cent resection of the acid secreting portion of the stomach was removed. Gastro-intestinal continuity was restored by either a Billroth I or II procedure (short loop posterior gastro-enterostomy).

Group II A. Sixteen dogs in whom a sleeve resection of the stomach was done, removing 50 per cent of the acid secreting area and anastomosing the residual gastric pouch to the antrum.

B. In 15 dogs the extent of resection was similar to II A but a "pie" or "wedge" was excised, leaving the lesser curvature intact.

Group III. This group consisted of 3 dogs of group II A and 3 of group II B who failed to develop histamine-induced ulcer after 30 days. These [illegible] ected, and gastro-intestinal

[illegible] lbs. were used. Nembutal injected intravenously was used as the anesthetic agent. An open type of anastomosis was done using shoe lace shod clamps placed across the cardia and antrum to control bleeding and spillage. Suture material consisted of a running inner catgut (3-0 chromic) layer for mucosal approximation and an outer layer of interrupted 4-0 Deknatel silk in the Lembert fashion for

* From the Department of Surgery, Cedars of Lebanon Hospital and University of [illegible]
The research herein reported was sup- [illegible] Memorial Research Fund and Mr. and

THE RELATIVE EFFECTS OF DIFFERENT GASTRIC DRAINAGE PROCEDURES ON THE HORMONAL PHASE OF GASTRIC SECRETION*

HENRY N. HARKINS, RALPH K. ZECH, LLOYD M. NYHUS, HORACE G. MOORE, JR., LESTER R. SAUVAGE, AND CHARLES A. GRIFFITH

Gastric drainage procedures are accepted as a necessary accompaniment of vagotomy. The use of such procedures obviates some of the postoperative difficulties, especially the gastric distention, which follow simple vagotomy.[1] At the same time, recurrence of ulcer has resulted after vagotomy and gastrojejunostomy, alone or in combination. Previous reports by our group before the Surgical Forum have indicated that both vagotomy[2] and gastrojejunostomy,[3] again alone or in combination, are a definite stimulus to Heidenhain pouch secretion. It was concluded from our previously reported studies that such a stimulation of the hormonal phase of gastric secretion might help to explain the occasional recurrent ulcer noted after these procedures.[4]

In an attempt to find another type of drainage procedure which would not produce a stimulating effect on the hormonal phase of gastric secretion, we studied Finney pyloroplasty.[5] In a report given before the 1953 Surgical Forum, it was shown that Finney pyloroplasty has essentially no stimulating effect upon the hormonal phase of gastric secretion and that Finney pyloroplasty, when used in conjunction with vagotomy, effectively maintains Heidenhain pouch secretions at near control levels.

The present paper summarizes an extension of our work to include not only gastrojejunostomy and Finney pyloroplasty, but also six additional drainage procedures, a total of eight. The entire list of operations includes three types of gastroduodenostomy (Jaboulay, end-to-side, and side-to-end), three types of pyloroplasty (Rammstedt pyloromyotomy, Heineke-Mikulicz, and Finney), and two types of gastrojejunostomy (standard posterior gastrojejunostomy and antrojejunostomy).

METHOD

Heidenhain pouches were formed in 60 dogs varying from 10 to 30 kg. in weight. The details as to animal care, pouch secretion collection and calculation of secretion as mEq of free HCl in 24 hours have been presented elsewhere[6] An adequate test period was considered to be 30 consecutive 24 hour collection periods. After a control period of 30 days, the drainage procedure was performed, the animal was allowed to completely recover from the operation and then a second observation period of 30 days of pouch collections was completed. Some animals had several procedures done, each separated by a 30 day period of pouch secretion data The stomata averaged 4 cm. in length at operation. Barium studies were made of representative animals in each of the eight series of experiments.

* From the Department of Surgery, University of Washington School of Medicine, Seattle This work was supported in part by Grant-in-Aid G-3542, National Institutes of Health, and by Initiative 171 Funds, State of Washington

juice from a Pavlov pouch. Dragstedt and co-workers[2] have demonstrated clearly that the stimulating effect of antral exclusion on a Heidenhain pouch in dogs is distinctly less if a small remnant of gastric acid secreting mucosa is left attached to the excluded antrum Furthermore, these authors observed that the application of acid to an isolated antral pouch could inhibit the acid secretion of an isolated gastric pouch occasioned either by distention or the application of food into the antral pouch.

From our results as well as the findings of other investigators we can assume that when the antrum is in continuity with the acid secreting portion of the stomach, as the secretion of gastric juice increases, inhibition of gastric acidity comes into play. Whether this inhibition is a specific function of the antrum and, if so, whether it is due to decreased formation of gastrin or to the liberation of a specific gastric inhibiting hormone, is not yet known. Further studies to elaborate these facts are at present in progress in our laboratory.

SUMMARY AND CONCLUSION

In dogs in which 50 per cent of the acid secreting portion of the stomach has been removed, the incidence of histamine induced ulcer is significantly less when the antrum is preserved and anastomosed to the residual gastric pouch than when the antrum is excised and gastro-intestinal continuity is restored by Billroth I or II technique. Of six dogs who failed to develop a histamine induced ulcer with a 50 per cent "sleeve" or "pie" resection, five did so when the antrum was resected and continuity restored by the Billroth I or II technique The amount of residual and secreting gastric mucosa remained the same, before and after antral resection.

The possible mechanism by which this protective action of the antrum in continuity is exercised, is discussed.

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particular procedure involved as compared with the 30 day basic control secretion period.

Main credence is given to the results following primary operations. The animals remained in such good nutritional status that many of them had multiple surgical procedures performed in sequence and it was possible to transfer some animals from one series to another. These results are included in smaller print. It is felt that they are of interest although the results were more variable, except after vagotomy, than after the primary procedures, possibly because a small portion of stomach, duodenum, or jejunum was of necessity destroyed each time an anastomosis was taken down.

Gastroduodenostomies. The results were somewhat variable, but in general indicated some stimulating effect, being 50 per cent, 12 per cent and 34 per cent, respectively (average +32 per cent) in the three types. In general the procedures which involved anastomosis of the duodenum to the antrum or body of the stomach produced more stimulation. None of these effects are as marked as that following standard gastrojejunostomy (+167 per cent) and none of the effects in this group associated with vagotomy were as marked as those of vagotomy alone[3] (226 per cent).

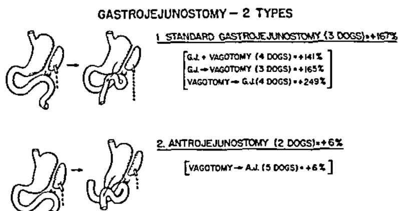


Fig. 3. Summary of results following two types of gastrojejunostomy.

Pyloroplasties. These demonstrated a consistent inhibitory effect on Heidenhain pouch secretion, or at least there was no stimulating effect following the primary operations (average —13 per cent).

Gastrojejunostomies. Here the opposite pertained and a consistent marked stimulatory effect was noted (average +87 per cent).

Barium studies indicated good drainage of the stomach except in the case of the Jaboulay gastroduodenostomy and the standard gastrojejunostomy, where a "circus movement" of the barium was noted. When simultaneous vagotomy was performed with the other drainage procedures, drainage was as good as with gastrojejunostomy.

DISCUSSION

The factors which may explain the stimulatory effect of gastrojejunostomy upon the hormonal phase of gastric secretion as evidenced by Heidenhain pouch output have been previously discussed[3] These are increased antral stimulation,[7] alkaline-acid rebound phenomenon, decreased duodenal acid inhibition,[8] and increase in the intestinal phase. Space does not permit an

RESULTS

The results are portrayed under three headings: *Gastroduodenostomies* (Fig. 1), *Pyloroplasties* (Fig 2), and *Gastrojejunostomies* (Fig. 3). Included with the Finney pyloroplasties and the standard gastrojejunostomies are those reported by Nyhus et al. at the 1953 Surgical Forum last year, but

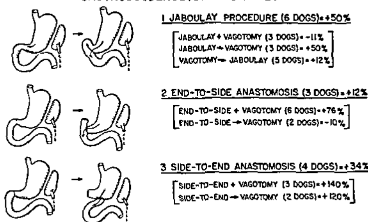


Fig. 1 Summary of results following three types of gastroduodenostomy. The procedure is depicted on the left. The results expressed as per cent change in free HCl output

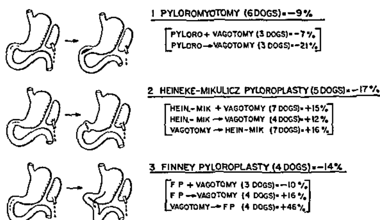


Fig 2 Summary of results following three types of pyloroplasty.

the animals included in our other previous reports are not included. This lack of inclusion of the previous animals is because (*a*) the controls were different, and (*b*) the results agree with those in the present experiments anyway. In each figure the procedure is graphically portrayed on the left and the results summarized on the right. The results are shown as average percentage change in mEq. free HCl in the observation period after the

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A MECHANISM FOR THE POTENTIATION OF GASTRIC SECRETION BY ACTH*

P. J. GERITY, J. A. CAMILLERI, AND M. A. HAYES

Surgical literature abounds with reports of the reactivation or development of peptic ulcers in patients while under therapy with adrenocortical or adrenocorticotrophic (ACTH) hormones. The mechanism of increased gastric secretion during hyperadrenocorticism is not clear; accordingly, it seemed of interest to plan a carefully controlled study in an effort to clarify some of the aspects of gastric secretion in relation to the pituitary-adrenocortical axis This study is limited to the effects of ACTH on gastric secretion

MATERIALS AND METHODS

Trained adult mongrel dogs ranging from 7 to 10 kilograms in body weight were prepared under pentobarbital anesthesia, using endotracheal positive pressure when the left pleural cavity was opened. Careful, sterile technique was observed throughout Four groups of dogs were prepared with isolated gastric pouches as illustrated in Figure 1. The pouches were of two types (1) with complete vagal denervation and, (2) with intact vagal innervation. When the operation of denervation was performed both vagi were divided supradiaphragmatically One group of animals had the gastric antrum left intact, while another group was subjected to resection of the antrum Postoperatively procaine penicillin was given. The dogs were supplemented with parenteral fluids and electrolytes as indicated. Sufficient convalescent time was allowed to insure the healthy condition of each animal. Collections of gastric secretions were obtained through a specially prepared plastic cannula connecting the gastric pouch to the outside.

Basal collections were taken over a 5 hour period, at the same time daily, under identical conditions A standard subcutaneous injection of histamine 5 mg. was used, and specimens collected on all dogs. As a control for the completeness of vagotomy, insulin was given intravenously to produce hypoglycemia[1] as measured by blood sugar levels.

Adrenocorticotrophic hormone was given by intravenous drip in doses of 30 mg. in 200 cc of 5 per cent glucose in water. The evening prior to this, a

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analysis as to how these various factors might act differently, but it would seem that the pyloroplasties would maintain the normal situation more than would the gastrojejunostomies, in conformity with our results Procedures such as standard gastrojejunostomy and the Jaboulay gastroduodenostomy allow a repetitous stimulation of the antral mucosa with food, and of the mucosa of the body of the stomach with fluids of reduced acidity. Such action would have a tendency to promote both antral activity and alkaline-acid rebound, again in conformity with our results. True, these two procedures afford adequate gastric emptying following vagotomy, yet their disadvantages as far as acid stimulation is concerned counterbalance this apparent advantage. Pyloroplastic procedures, on the other hand, do not allow repetitious antral stimulation, reduce alkaline-acid rebound, and intensify duodenal inhibition. While they may increase intestinal stimuli, these latter are considered to be of minor importance in regulating [illegible] It would seem, therefore, that [illegible] duodenostomy are ph[illegible] tic procedures which [illegible] to a large extent, at least in the [illegible] vagotomy, clinical experience has borne out the soundness of this reasoning

SUMMARY

1. Certain gastric drainage procedures would seem to be physiologically more sound than others in preventing an increase in the hormonal phase of gastric secretion, both with and without vagotomy

2 Experiments were performed on 60 dogs testing the effects of three groups of drainage procedures (8 individual types of operation) as measured by the effects on free HCl output from Heidenhain pouches.

3. A correlation was found in that procedures which would seem to increase antral stimulation and alkaline-acid rebound, at the same time caused an increased secretion of HCl from Heidenhain pouches.

4. The pyloroplasties (average 13 per cent *decrease* in Heidenhain pouch secretion) seemed to be the most advantageous drainage procedures from the standpoint of lack of stimulation of the hormonal phase of gastric secretion The gastroduodenostomies (average 32 per cent *increase*) were in the intermediate position, and the gastrojejunostomies (average 87 per cent *increase* in Heidenhain pouch secretion) seemed to be the least advantageous

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The volume of all specimens was measured, and analyzed for free, combined and total acidity.

RESULTS

The findings are recorded in degrees of free acid as percentage of increase or decrease as compared to the basal (fasting) specimens (Table 1).

Group I: Pouch Prepared with Antrum and Vagus Intact. The fasting free acid was at a level generally considered to be normal. After histamine, there was a 50 per cent increase in the free acid as recorded in degrees of acidity. After insulin, there was a 20 per cent increase. After ACTH, there was a 61 per cent increase.

Group II: Pouch with Vagus Removed and Antrum Intact. The fasting free acid was within a normal range, but there was a small volume of secretion. After histamine, there was a 50 per cent increase in acid content. After insulin, a 41 per cent decrease below the basal level was noted. After ACTH, there occurred a 35 per cent increase in acidity.

Group III: Pouch with Antrum Resected, Vagus Intact. The fasting specimen showed no free acid. After histamine, there was a 136 per cent increase. After insulin there was a 60 per cent increase in acidity. After ACTH a 24 per cent decrease occurred.

Group IV: Pouch with Antrum Removed and Vagus Removed. The fasting specimen showed no free acid After histamine, there was a 110 per cent increase in acidity. After insulin and ACTH, there was no free acid found.

CONCLUSIONS

1. The adrenocorticotrophic hormone is a powerful stimulant for the production of free hydrochloric acid. In order for it to function in this capacity antral mucosa must be present.
2. ACTH has no hormonal effect either directly or through the adrenocortical glands on the isolated gastric pouch as measured by acid secretory response.
3. There is no evidence to indicate that the acid secretory effect of ACTH is mediated through the vagus nerve.
4. ACTH apparently stimulates the gastric antral tissue selectively to produce its "hormone," with subsequent hyperacidity, either directly or through a secondary response mediated through the adrenocortex; the latter route would seem more probable.

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preparatory dose of 30 mg. of ACTH was given intramuscularly. The dogs had been trained to stand or lie quietly for the 5 hour period required to collect the specimens. Eosinophil counts done before and after the injection demonstrated a satisfactory adrenocortical response.[2] All counts showed more than 50 per cent decrease in circulating eosinophils.

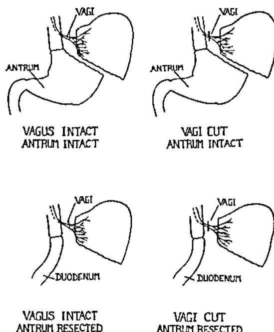


Fig 1 Diagrammatic illustration of types of gastric pouches prepared in the four groups of experimental animals

Table 1. Free Acid as Percentage Change

DOG GROUP	BASAL	HISTAMINE	INSULIN	ACTH
I Vagus + Antrum +	Normal	50% Increase	20% Increase	61% Increase
II Vagus — Antrum +	Normal (low volume)	50% Increase	41% Decrease	35% Increase
III Vagus + Antrum —	No free acid	136% Increase	60% Increase	24% Decrease
IV Vagus — Antrum —	No free acid	110% Increase	No free acid	No free acid

Results are indicated in degrees of free acid (HCl) percentage increase or decrease, as compared to basal specimens

1. The segment of duodenum, containing the pancreatic and bile duct orifices, is transplanted into the colon or into the terminal ileum not more than 75 cm. proximal to the ileocecal valve.

2. The dog survives postoperatively from six to twelve weeks. If bile salts and pancreatin are given as a postoperative supportive measure, a longer survival period is required for the ulcer to develop.

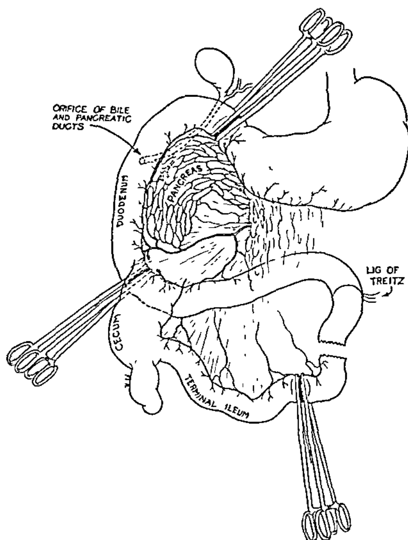


Fig 1. Surgical procedure, part I

PROCEDURES

The study presented here covers four series of dogs. These series were compiled by two of the authors, working independently, as controls on each other's results. The data of each are identical in the major aspects presented in this paper.

Series I. An unselected group of ten dogs, which meet the two criteria for ulcer production by terminal ileum implantation, are shown in Table I. One of the authors (D M.H.) fed bile salts and pancreatin to some of the dogs in his group when the animals appeared to be in critical condition

FURTHER STUDIES OF EXPERIMENTAL GASTRIC AND DUODENAL ULCERS IN DOGS*

EDWARD B. C. KEEFER, DANIEL M. HAYS, KIRBY A. MARTIN, JOHN M. BEAL, AND FRANK GLENN

The study of gastro-intestinal ulceration in the dog is extremely difficult to evaluate The variety in methods of creating ulcers suggests several possible causes, which may be classified as.

1 Neurogenic (vagus)
2 Humoral (pyloric antrum)
3 Secretory (gastric glands)
4. Indirect (bile and pancreatic secretions)

Table 1. Results in Series 1

DOG	DISTANCE PROXIMAL TO ILEOCECAL VALVE OF ORIFICES IN DUODENAL IMPLANT	POSTOPERATIVE SURVIVAL PERIOD	ULCERS	DUODENAL	GASTRIC
634	30 cm	73 DAYS	+	2	0
664	60 cm	60 DAYS	+	1	0
731	75 cm	75 DAYS	+	1	1
158	25 cm	69 DAYS	+	1	0
185	25 cm	48 DAYS	+	1	0
199	25 cm	56 DAYS	* ?	QUESTION OF EARLY ULCER	0
176	45 cm	85 DAYS,	+	2	2
190	50 cm	70 DAYS	* 0	0	0
139	50 cm	73 DAYS	+	2	0
167	75 cm	47 DAYS	* 0	0	0

* DOGS FED BILE SALTS AND PANCREATIN POSTOPERATIVE AS SUPPORTIVE MEASURE

For the past thirty years, the Mann-Williamson dog has strongly influenced ulcer research. The M.-W method employs not only the first three of the above ulcerogenic considerations, but also unaccustomed jejunal mucosa and an abnormal stoma. A surgical method of producing duodenal and gastric ulcers in dogs, without significant distortion of the gastro-intestinal tract, was presented at the 1953 Surgical Forum.[1] It was hoped that this method would offer a simple, easily controlled, basic procedure to replace the M.-W. dog. This surgical procedure has proven to be consistent in producing ulcers in dogs, provided that

* From the Departments of Surgery and Medicine of the New York Hospital-Cornell Medical Center. This research was aided by grants from the Doctor William Malcolm Fund and Mrs Percy Straus

carried out on these dogs; i.e., the segment of duodenum, containing the bile and pancreatic duct orifices, is isolated and a loop of terminal ileum, 75 cm. or less from the ileocecal valve, is brought up and an end-to-end, isoperistaltic anastomosis performed.

Series 2. This series consists of ten dogs with postoperative observations from six months to two years (Table 2). In these animals, the entrance of the bile and pancreatic orifices in the duodenal implant was placed 75 cm. or more proximal to the ileocecal valve (Figs. 1 and 2). Ulcers did not occur.

Series 3. Transplantation of the distal common bile duct into the terminal

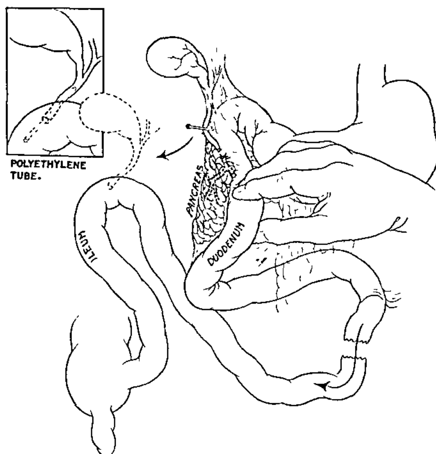


Fig 3 Common bile duct transplantation into terminal ileum procedure.

ileum (Fig. 3), 10 to 25 cm proximal to the ileocecal valve, did not produce ulcers in eleven dogs. This series was observed for a postoperative period of eighty-five days to seven months (Table 3). Several methods of implantation of the common bile duct, with and without indwelling plastic tube, were used The distal common bile duct was isolated at its entrance into the duodenal wall. All other structures, except the blood supply to the duodenum, were severed to prevent accessory bile ducts being overlooked.

Series 4. The common bile duct was isolated, as in series 3. The duodenal segment, containing the pancreatic duct orifices, was inserted into the terminal ileum 50 cm or less proximal to the ileocecal valve The common bile duct was then reimplanted into its original position in the repaired duode-

during the postoperative period. This may account for the failure of dogs Nos 199, 190, and 167 to develop definite ulcers. Figures 1 and 2 are reproduced from the 1953 Surgical Forum to illustrate the surgical procedure

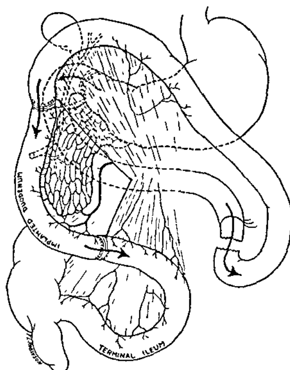


Fig. 2 Surgical procedure, part II

Table 2 Results in Series 2

DOG	DISTANCE PROXIMAL TO ILEOCECAL VALVE OF ORIFICES IN DUODENAL IMPLANT	POSTOPERATIVE SURVIVAL PERIOD	ULCERS
542	90 cm	2 YEARS	0
934	100 cm	1½ YEARS	0
927	100 cm	1 YEAR	0
82	80 cm	11½ MONTHS	0
96	150 cm	11 MONTHS	0
108	290 cm	10½ MONTHS	0
129	280 cm	8 MONTHS	0
142	100 cm	7½ MONTHS	0
175	150 cm	6¾ MONTHS	0
259	75 cm	6 MONTHS	0

num (Fig. 4). Insufficient studies on this group precluded any preliminary report.

OBSERVATIONS

Series 1. The duodenal segment, containing the bile and pancreatic duct orifices, was transplanted into the terminal ileum at a specific site, measured proximally from the ileocecal valve. This series suggested, in order to



Fig 5 Experimentally produced perforated duodenal ulcer and associated proximal duodenal ulcer (E B C K)



Fig 6. Experimentally produced duodenal ulcers (D.M H). Note. Ulcer not associated with suture line.

Table 3. Results in Series 3

DOG	DISTANCE PROXIMAL TO ILEOCECAL VALVE OF REIMPLANTATION OF COMMON BILE DUCT	POSTOPERATIVE SURVIVAL PERIOD	ULCERS
220	15 cm	85 DAYS	0
1	10 cm	3 MONTHS	0
2	15 cm	3 MONTHS	0
3	15 cm	3 MONTHS	0
154	15 cm	3½ MONTHS	0
229	25 cm	4 MONTHS	0
237	25 cm	4⅓ MONTHS	0
241	25 cm	4¾ MONTHS	0
251	25 cm	6 MONTHS	0
239	20 cm	6½ MONTHS	0
223	25 cm	7 MONTHS	0

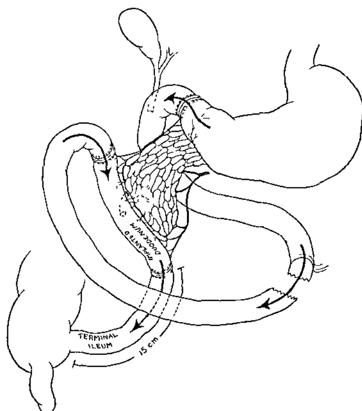


Fig 4. Pancreatic duct transplantation into terminal ileum procedure.

tal studies in dogs were done by McCann[5] using the Schmilinsky operation. He reported an 80 per cent incidence of stomal ulcer following this procedure. Other investigators,[6-8] however, reported a much smaller incidence of ulcer. Merendino et al.[9] carried out a study to explain these discrepancies and found that the length of the afferent duodenojejunal loop appeared to be the crucial factor in determining the frequency of ulceration using the Schmilinsky operation. Kesavalu and Mann[10] noted an increase in volume and acidity of gastric juice secreted from a Heidenhain pouch dog after total intragastric regurgitation of duodenal content. Dragstedt et al.[11] similarly reported an increase in acid production from 4 Heidenhain pouch dogs following the Schmilinsky procedure and a much greater increase when the animals were converted to a Mann-Williamson procedure. They attributed the increase in secretion to loss of acid-inhibition in the duodenum.

Since these studies have all entailed an operative procedure whereby the duodenal loop is excluded from contact with the ingested food, it seemed

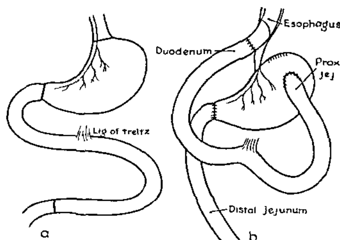


Fig 1 Stomach transplantation to 60 cm beyond the ligament of Treitz with preservation of the vagal innervation to the stomach The solid lines indicate the various sites at which the gut was transected

worthwhile to study this problem further, avoiding the exclusion or defunctionalization of the duodenojejunal loop produced by the Schmilinsky procedure.

METHOD

Operations were performed in healthy adult mongrel dogs using pentobarbital anesthesia and standard sterile technique. The transthoracic approach was utilized, entering the left tenth interspace with the animal in the lateral position. The membranous tissue at the esophageal hiatus was incised widely and usually the incision was extended about 5 cm. toward the central portion of the left diaphragm. This allowed the stomach to be delivered easily into the thorax The vagus nerves were carefully preserved. The esophagus was then divided just at the cardia and the distal end was closed blindly. Next the pyloric ring was divided and an end-to-end esophagoduodenostomy was performed. Finally the jejunum was divided 60 cm. beyond the ligament of Treitz, the proximal end was anastomosed end-to-side high on the anterior wall of the stomach at the level of the cardia, the

create an ulcer, the entrance of the bile and pancreatic secretions should be inserted into the terminal ileum near the ileocecal valve. The small intestine varies considerably in length, according to the size and species of the dog Regardless of this variation, a segment of terminal ileum of 75 cm. or less, rather than a percentage of the entire small bowel, permitted the ulcer to develop (Figs. 5 and 6).

These dogs were used for the purpose of measuring the regurgitation of bile and pancreatic secretions and for evaluating the neutralizing effect of these secretions on the gastric acidity. During exploratory operation, regurgitation was observed proximally for a short distance. However, these studies did not take into consideration such factors as food content, feeding conditions, and anesthesia.

Series 2. With long term survival of the dogs in this series, it was noted that the interrupted silk sutures eroded through to the mucosal surface. Finally, the silk became free in the intestinal contents. In no case did perforation or ulceration occur at the suture line. In spite of several incidences of extreme malnutrition and diarrhea with electrolytic imbalance and multiple pregnancies, producing a severe anemia, no ulcers were found in these animals. C [illegible] of termina [illegible]

Series 3 [illegible] ulcer production No ulcers occurred, even though these dogs were observed for a much longer postoperative period than those in series I.

Series 4. This modification of our method of ulcer production is being used for further studies to evaluate the role of the pancreatic secretions.

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THE EFFECT OF TRANSPLANTATION OF THE STOMACH TO THE LOWER JEJUNUM WITH PRESERVATION OF THE VAGAL INNERVATION*

WILLIAM D. KELLY, ALAN P. THAL, AND OWEN H. WANGENSTEEN

It has long been known that hydrochloric acid can inhibit gastric secretion when instilled in adequate concentration into the stomach or duodenum. Recently interest has been revived in acid-inhibition of the antral phase of gastric secretion by Dragstedt[1, 2] and others.[3] This work suggests that loss of acid-inhibition may be an important factor in the genesis of peptic ulcer after operations which exclude the gastric antrum from the remainder of the stomach. An operation which in effect excluded the duodenum was first suggested by Schmilinsky[4] as a means to treat peptic ulcer in humans. The operation proved to be, if anything, ulcerogenic. Subsequently, experimen-

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described previously. Following recovery from this operation for a minimum period of 3 weeks, the animals were again standardized by collection of the Heidenhain pouch secretion.

RESULTS

The results are tabulated in Table 1 and Figures 2 and 3. Briefly, where operation was followed simply by observation it was found that of 10 dogs surviving beyond 30 days, 5 developed ulceration in the jejunum just beyond the anastomosis to the stomach, 4 of these ulcers being perforated. Of the other five animals, two died apparently of acute gastric dilatation, one died

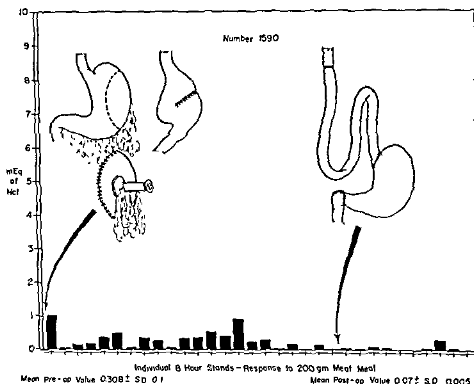


Fig 2 Acid output in mEq from a Heidenhain pouch before and after gastric transplantation Dog 1590 died of a perforated stomal ulcer the day after the last figure illustrated.

of pneumonitis, one died of bowel obstruction secondary to adhesion formation and one died of cachexia of undetermined origin.

Two animals of the latter 5 had had antrectomy done 6 months after the initial operation, at which time no ulcer was found. They survived the second operation 78 and 81 days, respectively, dying without the occurrence of an ulcer.

Data are available on only two dogs in which Heidenhain pouches were created. Both animals were in good physical condition at the time of the pouch collections. Dog 1590 showed an average secretion of 0 308 ±0.10 mEq. of free acid following a 200 gram test meal of horse meat before transplantation and 0 070 ±0 005 mEq. following transplantation. The first figure is based on 20 stands, the second figure on only 7 stands since the animal died of a perforated jejunal ulcer the day following the last stand.

distal end was anastomosed end-to-end to the lower end of the stomach (Fig 1). Particular care was taken to obtain a wide lumen at the anastomoses by obliquing the cut where necessary and by avoidance of a large cuff. The stomach and bowel were carefully returned to the abdomen and the diaphragm was closed about the esophagus above the level of the esophago-
tion, penicillin in oil and beeswax
intravenous fluids were given for
milk and water only for several
more days and then fed a standard diet of commercial dog kibbles supplemented with 1 pint of milk and 1 can of commercial dog food daily. One group of dogs was simply followed for a minimum of 6 months. It was proposed to perform a second operation at the end of this period, at which time resection of the antrum and several inches of jejunum beyond the

Table 1 Stomach Transplant to 60 cm. beyond the Ligament of Treitz with Preservation of Vagi

ANIMAL NO		LENGTH OF SURVIVAL AFTER OPERATION	REMARKS
1	842	261 days	Reoperated after 6 months, antrum excised, no ulcer Died 81 days later. Distended stomach (5 × normal) only apparent cause —no obstruction
2	1164	100 days	Perforated jejunal ulcer
3	1040	69 days	Acute gastric dilatation. No ulcer or erosion No obstruction
4	1299	104 days	Perforated jejunal ulcer
5	1387	49 days	Purulent nasal discharge, patchy consolidation in lungs, no ulcer
6.	1502	190 days	Marked emaciation No ulcer All anastomoses adequate Partial narrowing of CBD from stitch and scar No jaundice
7	1591	270 days	Antrectomy done 9 months after transplant No ulcer Died 78 days later of bowel obstruction secondary to adhesions. No ulcer
8	1603	74 days	Perforated jejunal ulcer
9	61	53 days	Perforated jejunal ulcer
10	188	91 days	Multiple jejunal ulcers

gastrojejunal anastomosis would be done followed by end-to-end reconstitution of continuity
the creation of a Heidenhain
pc steel outer sheath similar to
th eeks following recovery from
the operation the animals were standardized by collection of 24 hour outputs from the pouch as well as in response to a test meal The animals were fasted for 16 hours prior to the test meal which consisted of 200 grams of cooked horsemeat. Two fasting specimens of one hour duration each were collected first. The test meal was then fed and 6 additional one-hour specimens were collected. A minimum of 10 stands and 20 overnight collections were done. The volume of juice was recorded, the free and total acidities were determined using the standard titration technique with Topfer's reagent and phenolphthalein for indicators After standardization the animals were subjected to transplantation of the stomach to mid-jejunum as

of the gastric juice by bile, pancreatic juice and succus entericus and an increased susceptibility of the bowel to ulceration as one progresses downward have been considered to be likely factors.

The occurrence of acute gastric dilatation is of some interest since it was observed in two dogs, occurring after a long interval following operation. These animals were both apparently in good health and eating well the day prior to their sudden demise. At autopsy an enormous distention of the stomach was found, estimated at five to eight times the normal size. The stomach wall was paper thin. The content was largely gas with only a small amount of liquid material present. No cause for the distention of the stomach could be found. Acute gastric dilatation was seen a number of times immediately after the stomach transplantation operation. However, these two animals had survived operation for a period of 69 days and 261 days, respectively, when gastric distention suddenly occurred apparently without cause. Postoperative acute gastric dilatation has been generally attributed to a reflex paralysis of the stomach secondary to operative manipulations. Obviously, this cannot account for the two cases described here. Kinking of the gastric outlet does not seem to be a feasible explanation since this would produce signs of obstruction with prolonged vomiting and refusal to eat. Moreover, the degree of gastric distention seen after mechanical obstruction does not approach the enormous distention occurring with acute gastric dilatation It may be conjectured that the altered anatomy of the upper gastro-intestinal tract following stomach transplantation in some unknown way might perpetuate or accentuate an otherwise normal inhibitory reflex acting to delay gastric emptying.

SUMMARY

The effect of transplanting the vagally innervated stomach to a point in the jejunum 60 cm. beyond the ligament of Treitz was studied. Peptic ulcer occurred in the jejunum just beyond the gastric stoma in 5 of 10 dogs observed for periods ranging from 49 to 270 days. Four of the five ulcers were perforated Studies of gastric secretion made in two Heidenhain pouch dogs before and after stomach transplant failed to reveal an increase in gastric secretion, thus suggesting that decreased neutralization of acid or increased susceptibility to ulceration are the responsible factors. Acute gastric dilatation occurring spontaneously in two dogs long after operation was an incidental finding.

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Dog 1790 secreted 1.110 ± 0.170 mEq. of free acid from his Heidenhain pouch prior to transplantation and 0.527 ±0.100 mEq. following transplantation These figures are based on 20 and 19 stands, respectively. The overnight collections which represent the secretion occurring during the remaining 16 hours of the 24 hour period following an 8 hour stand showed a much more variable secretion from day to day. However, there was no appreciable change in the values obtained before and after stomach transplantation.

DISCUSSION

The findings obtained from the Heidenhain pouch studies appear to be contradictory to the results obtained by previous investigators Obviously, the pouch data reported here are not to be considered conclusive in view of

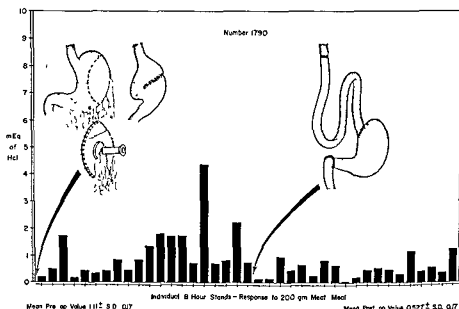


Fig 3 See Figure 2 for legend.

the small number of animals studied and the well known variability of gastric secretion. Further observations must be made. Secondly, the operative procedure used in this report differs from the classic Schmilinsky procedure in causing the ingested food, water and saliva to pass through the duodenojejunal loop leading to the stomach. No conclusions as to the underlying differences are possible at present. The occurrence of a perforated ulcer in the jejunum just beyond the gastric outlet in the absence of any increase in secretion from a Heidenhain pouch (dog 1590) certainly suggests that hypersecretion is not playing a role in the genesis of this particular type of experimental ulcer. It is difficult to believe that hypersecretion of cephalic origin could result from the operative procedure described here. The other major alternative which has received considerable attention from previous workers is the theory that ulceration may develop in the presence of a normal gastric secretion owing to a decrease or loss of the local defense mechanisms of the bowel against peptic ulceration Decreased neutralization

Twenty-one such collections, not necessarily on consecutive days, were made on each of the 13 dogs before the antrum was operated on.

After these control data had been obtained, the antrum in each case was detached from the stomach with care to preserve its blood supply, its proximal end was closed and its distal end was anastomosed with the antimesenteric border of the colon about 15 cm. beyond the ileocecal junction. Gastro-intestinal continuity was re-established by an end-to-side gastroduodenostomy Beginning about 1 month after this operation, the series of

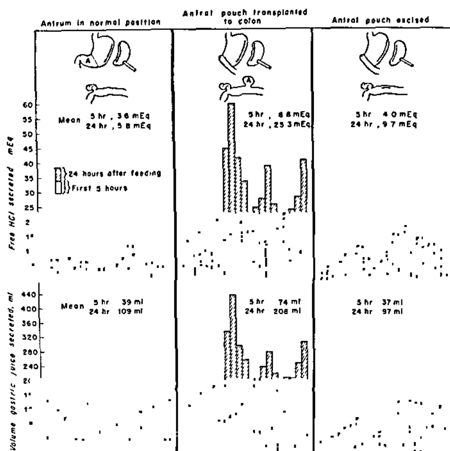


Fig. 1 Data from a typical dog before and after transplantation of the antrum to the colon and after excision of the antrum

twenty-one daily collections of gastric juice from the Heidenhain pouches was repeated.

Twelve dogs survived these procedures In 6 of these, the antral pouches were then detached from the colon and made to drain to the exterior. In the remaining 6 dogs, the antral pouches were removed. After the animals had recovered from these procedures, a third series of twenty-one collections of gastric juice was accumulated.

RESULTS

The volume of gastric juice and quantity of free hydrochloric acid secreted by a given Heidenhain pouch in response to the normal stimulus of feeding varied considerably from day to day. Figure 1 depicts the values

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THE EFFECTS OF EXCISING, EXTERIORIZING AND TRANSPLANTING THE PYLORIC ANTRUM TO THE COLON IN DOGS WITH HEIDENHAIN POUCHES*

JOHN D. BOTTI AND GEORGE A. HALLENBECK

For many years after Edkins and Tweedy[1] in 1909 postulated the theory that the pyloric antrum elaborated a hormone which stimulated the remainder of the stomach to secrete acid gastric juice, evidence that the antrum occupied this special role was inconclusive Recent studies, particularly the impressive experiments by Dragstedt and his colleagues,[2-5] have produced strong support for this concept

The present experiments were performed to restudy the effects of transplanting the pyloric antrum to the colon and of excising the antrum on the secretory responses of vagally denervated (Heidenhain) pouches in dogs.

METHODS

Gastric juice secreted in response to daily feeding of 400 to 600 gm. of Fromm's prepared dog food was collected from 13 dogs with Heidenhain pouches. The pouches were drained by vitallium cannulas to which rubber balloons could be attached. Early in the experiment, jackets made of a semirigid plastic material were fitted to the dogs to protect the balloons. Though this method is effective in keeping the animals from molesting the balloons, it also prevents them from keeping the skin around the cannulas clean As a result, maceration and excoriation of the skin occurred unless collections were limited to alternate days During most of the experiments, the balloons were left hanging unprotected and it was found that the dogs learned to leave them alone after a few trials. The gastric juice obtained was divided into that part secreted during the first 5 hours after feeding and that secreted during the following 19 hours The volume of each sample of juice was recorded and the quantity of free hydrochloric acid contained therein was determined by titrating an aliquot against 0.1 N sodium hydroxide, with Topfer's reagent being used to indicate the endpoint.

* Abridgment of thesis submitted by Dr Botti to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Master of Science in Surgery.

of the increase in total daily secretion was greater than that for the first 5 *hours after feeding.* The output of free hydrochloric acid increased to a greater extent than did the volume of juice secreted. Thus the mean 24-hour volume increased almost threefold from 67.9 to 191.1 ml. while the mean 24-hour output of free hydrochloric acid increased more than fivefold from 4.7 to 24.9 mEq Mean differences between rate of secretion and output of free hydrochloric acid when the antrum was transplanted to the colon and when it was in normal position or when it was excised or exteriorized were statistically highly significant.

COMMENT

Dragstedt and his colleagues[2, 3] have previously reported that transplantation of the pyloric antrum of the dog to the colon as a diverticulum causes Pavlov pouches, Heidenhain pouches and pouches of the entire remainder

Table I. Comparison of Gastric Secretion from Heidenhain Pouches in Dogs before and after Transplantation of the Pyloric Antrum to the Colon and after Removal or Exteriorization of the Antrum

		Mean ml. of gastric juice secreted during indicated period after feeding		Mean mEq. of free HCl secreted during indicated period after feeding	
	Dogs	First 5 hours	24 hours	First 5 hours	24 hours
A Antrum normal	13	25 2	67.9	2 6	4 7
B. Antrum transplanted to colon	13	58 4	191.1	7 7	24 9
C. Antrum excised or exteriorized	12	24 4	62.1	2 5	5.2
B–A		33.2 ± 6 26	123 2 ± 21 9	5 1 ± 1.01	20.2 ± 3 62
p		< 001	< 001	< .001	< .001
B–C		34 0 ± 8 88	129 ± 29 45	5 2 ± 1.31	19 7 ± 4.47
p		< 001	< 001	< 001	< .001
A–C		0 8 ± 4 79 (not sig.)	5 8 ± 13.24 (not sig)	0.1 ± .59 (not sig)	– 5 ± .96 (not sig)

of the stomach to secrete greatly increased quantities of gastric juice and free hydrochloric acid during a regimen of normal daily feeding The present data confirm this observation for Heidenhain pouches and further indicate that the phenomenon is demonstrable when the secretion occurring during the first 5 hours after feeding is considered as well as when the daily total secretion is measured. This experiment, together with studies by Dragstedt and his associates[2] in which the pyloric antrum was transplanted to the duodenum with similar results, is important in establishing the fact that the pyloric antrum of the dog, when properly stimulated, can exert a profound effect on the secretion of acid gastric juice by the remainder of the stomach

In the present experiments, whether the antral pouches were excised or separated from the colon but left in the body as a separate pouch draining externally, the volume and content of free hydrochloric acid of juice secreted during the first 5 or 24 hours subsequent to feeding returned to the same levels previously observed when the antrum was in its normal position. The

obtained from a typical dog before and after the pyloric antrum was transplanted to the colon and after the antral pouch was excised. Despite the rather wide fluctuations of individual values in any given category, the increase in both volume of juice and milliequivalents of free hydrochloric acid secreted after transfer of the antrum to the colon and the return to the previous level after the antrum was removed are evident.

Figure 2 summarizes graphically the data on all 13 dogs. A great increase is seen in both the volume of juice and the quantity of free hydrochloric acid secreted by the Heidenhain pouches when the pyloric antrum was

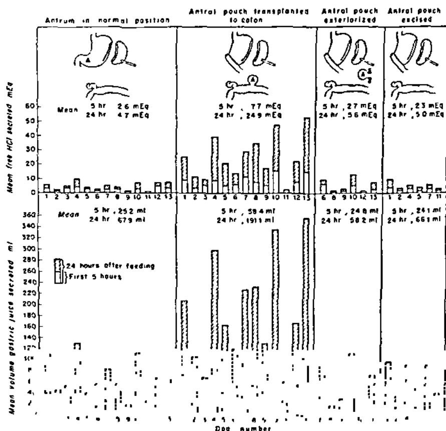


Fig. 2. Mean data for all dogs before and after transplantation of the antrum to the colon and after either excision or exteriorization of the antrum

attached as a diverticulum to the colon One of the 13 dogs died 55 days after transplantation of the antrum when a duodenal ulcer underwent free perforation. The antrum was excised in 6 of the remaining animals and in the others it was detached from the colon but preserved as a separate exteriorized pouch. It is evident from Figure 2 that secretion from the fundic pouches was not significantly different in these two groups of experiments and that it returned to the level observed before transplantation of the antrum.

The data are summarized in Table 1. The increased rate of gastric secretion after transplantation of the antrum to the colon was noted whether data for only 5 or for 24 hours after feeding were considered, but the magnitude

acid material. It is possible that diversion of a greater proportion of the acid-secreting gastric mucosa to form the pouch could prevent or delay the normal ultimate acidification of chyme passing through the antrum after feeding and thus enhance the effect of the antrum in stimulating the acid-producing mucosa to secrete. The factor of acidity cannot be the only one operating, or the 6 dogs of the present study in which the antral pouches were exteriorized and in which the *p*H was consistently neutral should have had increased secretion from the Heidenhain pouches, which was not the case. Perhaps some combination of alkaline *p*H with other chemical or mechanical stimulation associated with being in continuity with the gastro-intestinal tract is the adequate stimulus to make the antrum release its "gastrin." Whatever the cause might be, if the antral mechanism were making a greater contribution to the total stimulation of the pouches when less acid-secreting mucosa remained as part of the main stomach, antrectomy would be expected to cause a greater reduction in the secretion of such pouches than would be the case when the main part of the stomach was less altered in the course of making the pouch. The rather modest reduction in secretion from vagally innervated total stomach pouches after antrectomy, which led Dragstedt and his associates to suggest that it might be entirely accounted for by the incidental removal of some acid-secreting mucosa as well, could be explained on the same basis by assuming that in this case the antrum, never touched by food and continuously bathed in acid juice, was really contributing little to the secretion obtained before antrectomy.

With this hypothesis in mind, it does not seem unreasonable to suggest that the failure of excision or exteriorization of the antrum to alter secretion from Heidenhain pouches significantly in the present study may mean that the antrum does not normally play an important role in the stimulation of these pouches. The capacity of the antrum to do so under special circumstances is unquestioned.

This hypothesis could be tested experimentally in a number of ways and such studies might yield interesting results.

CONCLUSIONS

1 The observation of Dragstedt and his associates that transplantation of the pyloric antrum of the dog as a diverticulum of the colon results in greatly increased secretion from Heidenhain pouches is confirmed.

2. Under the conditions of these experiments, excision or exteriorization of the pyloric antrum in dogs does not significantly alter the secretion produced by Heidenhain pouches during normal daily feeding. A hypothesis is advanced to account for the variance between this finding and the findings previously reported by others.

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logical conclusion to be drawn from this result would be that despite the fact that the pyloric antrum was shown to influence greatly the secretion from a Heidenhain pouch under special circumstances, it does not play an important role in stimulating secretion from this type of pouch under normal circumstances. It should be noted, however, that Dragstedt and his associates[3] have observed a great decrease in secretion from Heidenhain pouches after antrectomy. They have described a group of 6 dogs in which they successively removed first one-third, then two-thirds and finally all of the

free hydrochloric acid from the pouches 53.7 mEq. when the antrum was intact pletely excised. Similar results were obtained in an almost identical study of 5 dogs reported by Helsby and Auten.[6] Antrectomy was found by Woodward and co-workers[4] to be followed by greatly diminished secretion from Pavlov pouches, and by Dragstedt and co-workers[5] to cause a considerably less marked fall in secretion from total gastric pouches with intact vagal nerve supply. The data are completely convincing and must be considered to represent the true state of affairs under the conditions of the experiments.

In an attempt to explain the discrepancy between our data and those cited above, let us examine some differences between the two groups of experiments. It does not seem reasonable that our results could have been influenced by the fact that excision or exteriorization of the antrum was preceded by a period of time during which the antrum was attached to the colon. It might be suggested that our results could have occurred if we had failed to remove enough of the antrum. Apart from the fact that we used the same landmarks described by other investigators, microscopic examination of the remaining portion of the stomach in some of the dogs showed that the rim of pyloric glands still present in some of them measured only from a few millimeters to at most a centimeter in depth. It seems fair to conclude that at least 90 per cent of the antrum was always removed, an amount which produced notable decrease in secretion from Heidenhain pouches in the experiments of others cited above.

One obvious difference between our experiments and the others mentioned is the fact that our Heidenhain pouches consistently produced less volume of juice and less free hydrochloric acid than those of the other investigators. In the study by Helsby and Auten,[6] the mean daily volume of juice secreted was approximately 280 ml. and the mean daily output of free hydrochloric acid was 34 mEq., comparable values in our series being 67.9 ml. and 4.7 mEq., respectively. The mean values in the study by Oberhelman and associates[3] were even higher, that for milliequivalents of free hydrochloric acid being 53.7. It seems likely that the other workers must have made pouches which were consistently larger than ours, though this must remain presumptive since we have had no way of checking the point. Granted this assumption, one would conclude that the other workers diverted a greater proportion of the acid-secreting portion of the stomach to form the Heidenhain pouch than we did unless the dogs used in the other studies were considerably larger than ours. Those of Helsby and Auten, at least, are described as weighing between 25 and 35 pounds (11.3 and 15.9 ...ghts similar to those in our series

the concept that the pyloric mucosa can n alkaline content but not when containing

and obliterate the esophageal lumen, thereby preventing reflux of gastric contents.

Two methods for construction of this papilla were used.

Group I. This procedure was similar in all details to the Mathisen technique employed in ureterosigmoidostomy (Fig. 1). This method was employed in 14 dogs. In the procedure a rectangular flap of gastric mucosa, based on one edge, was used as a covering for that portion of the esophagus which extended into the stomach lumen. However, because of a tendency

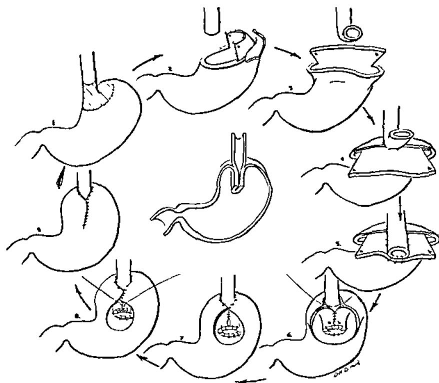


Fig. 1. *1,* The esophagogastric junction is excised as shown by the shaded area. *2,* A rectangular flap of gastric mucosa attached by one edge is created by excising the seromuscular layer. *3* and *4,* The posterior wall of the esophagus is sutured to the base of the flap. *5,* The free edge of the mucosal flap is sutured to the transected end of the esophagus and additional sutures are placed between the flap and the esophagus to obliterate dead space. *6* and *7,* The sides of the flap are sutured together and to the anterior surface of the esophagus. *8,* The gastric wall is closed partially and the end of the nipple sutured to the gastric wall. *9,* The gastric wall is closed in 2 layers. Center drawing shows cross section through stomach and esophageal valve.

of the papilla to invert under increased intragastric pressure, a single suture was used to suture the papilla to the inner gastric wall.

Group II. Dissatisfaction with the fixation by suture of the papilla to the inner gastric wall resulted in a major modification of the Mathisen procedure. Instead of cutting the lateral attachment of the mucosa-submucosal layers, these layers were left attached, consequently, when the esophagogastrostomy was performed, the intragastric portion of the esophagus was suspended from the stomach wall by a fold of gastric mucosa which contained the esophagus between its two leaves and thus suspended it in much

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THE SURGICAL CONSTRUCTION OF AN ESOPHAGEAL VALVE TO REPLACE THE "CARDIAC SPHINCTER"*

An Experimental Study

D. H. DILLARD, C. A. GRIFFITH, AND K. ALVIN MERENDINO

Inactivation of the "cardiac sphincter mechanism," regardless of the cause, frequently is followed by reflux peptic esophagitis and its associated complications of ulceration, bleeding, perforation and stricture. The surgical approaches to the problem encountered in esophageal reflux have been many Few, however, have been concerned with the actual need. While by-pass procedures[1] and measures directed toward a reduction in gastric acidity[2] are meritorious contributions, they represent indirect approaches to the problem It appears that gastroesophageal reflux is a secondary phenomenon. The primary difficulty is an incompetent sphincter. Therefore, surgical procedures might properly concern themselves with a direct approach, viz. the manufacture of a synthetic valve of autogenous tissue.

The material to be presented represents an attempt to construct and evaluate such a substitute cardiac sphincter in order to prevent gastroesophageal regurgitation.

METHODS AND MATERIALS

Twenty adult mongrel dogs were operated on through a left combined thoraco-abdominal or a left thoracic incision The distal 4 cm. of the esophagus and the proximal 4 cm of the stomach were excised A complete bilateral supradiaphragmatic vagotomy and a Rammstedt pyloromyotomy were done. An esophagogastrostomy was performed in the chest with approximately ⅓ to ½ of the stomach remaining above the diaphragm The diaphragm then was sutured about the stomach so that the new hiatus created was approximately the normal circumference of the stomach

The principle employed in the esophagogastric anastomosis was to extend the distal end of the esophagus with a covering of gastric mucosa into the lumen of the stomach so that a papilla at least 1½ inches long was made. Since fluid pressure is distributed equally to all surfaces in a closed system, any increase in intragastric pressure would serve to compress the papilla

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the 55th day postoperative) were subjected to chronic histamine stimulation by the injection daily of 30 mg. of histamine base in oil and beeswax intramuscularly for 45 days. Histamine phosphate was employed and each preparation was tested for potency on Heidenhain pouch dogs and on a control series of animals.

At autopsy sections for microscopic study were made of the esophagogastric anastomosis, 10 cm. up the proximal esophagus and elsewhere as indicated.

RESULTS

Because of the disparity in number between groups I and II, the combined results will be presented first (see Table 1).

Five (25 per cent) of the animals died in 14 days or less postoperatively. Two of these deaths were attributed to operative trauma; one died from diarrhea of unknown cause, one from empyema, and one died from atelectasis. Two animals died in 18 and 24 days respectively, due to stenosis of the valve An additional animal died 50 days postoperatively of an undetermined cause None of the esophagogastric anastomosis leaked into the chest or disrupted. It is probable that inversion of a large cuff of esophagus and stomach at the anastomosis greatly reduces this hazard.

In all, stenosis was encountered in 6 animals. It was the operator's impression that stenosis in 3 of these 6 cases was due to making the stoma too small at the time of surgery. However, in the remaining 3 cases, the stenosis appeared to be related to scarring and prolonged healing associated with esophagitis.

Twelve animals were begun on histamine in beeswax between 55 and 76 days postoperatively. Only one animal (No 18) showed esophageal inflammation by esophagoscopy prior to the addition of histamine, and this was noted on the 49th postoperative day. However, following histamine 10 (83 per cent) developed some degree of esophagitis In 2 animals this had progressed to the point of ulceration The remaining animals had esophageal inflammation and erosions only. There were no perforations of the esophagus. The stomach and duodenum, however, suffered greater damage, with 11 (92 per cent) showing some degree of inflammation This included 5 perforated gastric ulcers and 2 severe duodenal ulcers.

Valvular incompetence was considered to be present when it could be demonstrated by any one of the methods tried For example, dog No. 14, though competent by esophagoscopy, barium swallow, and at autopsy, was classified as incompetent when apomorphine-induced vomiting was carried out. By this rigid criterion, 9 of 20 animals had incompetent valves. Undoubtedly, apomorphine-induced vomiting represented the severest test Five of seven animals so tested were demonstrated to have incompetent valves. Incompetence was thought to be due to several factors. In 2 dogs the nipple was found at autopsy to be so short that for practical purposes no nipple effect existed. In 2 instances, there was separation of the nipple tip from the gastric wall and in 5, the suture which anchored the nipple to the gastric wall had drawn the gastric wall out, forming a band which was so long that for all practical purposes the valve was no longer anchored.

Although the number of animals coming to histamine stimulation in group II is small (4), some differences as contrasted with group I are of interest. All 8 (100 per cent) of the animals in group I receiving histamine

the same manner that the small bowel is supported by its mesentery (Fig. 2). The second method was used in 6 dogs.

The animals received penicillin and fluids by clysis for 2 to 3 days and then were begun on a special diet of Osterized milk and Friskies Dog Food

The ability of the valve to prevent reflux was tested in each animal by one or more of four different methods:

1. All animals were esophagoscoped and examined for evidence of inflammation or stenosis. A crude evaluation of valve competence was gained by passing the esophagoscope through the nipple and noting whether or not

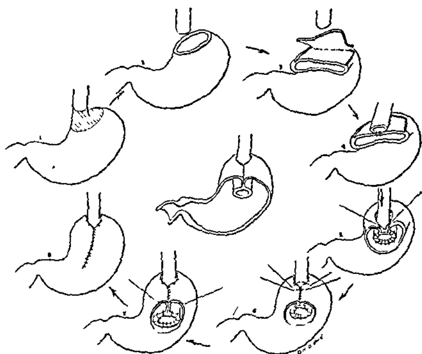


Fig 2 *1* and *2*, The esophagogastric junction is excised *3*, The seromuscular layer is dissected away from the mucosal flap *4*, The posterior wall of the esophagus is sutured to the flap *5*, The flap is sutured about the esophagus *6* and *7*, The sides of the flap [illegible] ...ls to hold the ... 2 layers and "mesentery."

the valve closed properly upon withdrawing the scope and noting whether [illegible]

[illegible] fluoros-

[illegible] ting and the character of the vomitus was noted. Alkaline frothy or mucoid material was seen in those animals with competent valves. Bile or acid material, of course, denoted an incompetent valve

4. F[illegible] were inflated with H_2O and any v[illegible] against an intragastric pressure o[illegible] competent.

Animals surviving 50 days or longer (one animal received histamine on

Group II	11	2	0	2	—	—	—	+	0	0	0	0	Operative shock
No histamine given (2 animals)	5	6	0	6	—	—	—	+	0	0	0	0	Empyema. No leakage
Histamine given (4 animals)	19	60	3	63	+12 +47	+12	+47	+	0	0	4	0	Perforated gastric ulcer
	18	61	9	70	+15 +49	—	0 49	+	0	2	4	0	Perforated gastric ulcer
	4	76	45	121	+ 9 28	+56	—	+	+	0	0	1	Sacrificed
	20	60	44	104	+46	—	+46	0	+	2	2	0	Sacrificed

• Competent Valve = +, incompetent Valve = 0 The time at which the test was performed is shown numerically in postoperative days.

† The severity of the pathology is graded on a gross and microscopic basis according to the following scheme: grade 1, microscopic inflammation only; grade 2, microscopic inflammation plus erosion not greater than 1 cm in diameter and not extending deeper than the superficial layers of the submucosa, grade 3, erosion greater than 1 cm in diameter or ulcers extending beyond the submucosa; grade 4, perforated ulcers.

Table 1. Combined Results of Experimental Construction of an Esophageal Valve

Operative Procedure	Dog No	Postoperative Survival Days: Prior to Histamine	Postoperative Survival Days: During Histamine	Postoperative Survival Days: Total Survival	Valve Competent as Tested by Various Methods*: Esophagoscopy	Barium Swallow	Apomorphine	H_2O Pressure	Stenosis	Gross and Microscopic Pathology†: Esophagus	Stomach	Duodenum	Comments
Group I	3	1	0	1	—	—	—	+	0	0	0	0	Death due to operative shock
	8	2	0	2	—	—	—	+	0	0	0	0	Atelectasis
No histamine given (6 animals)	12	14	0	14	—	—	—	—	0	0	0	0	Diarrhea, undetermined cause
	1	18	0	18	+16	—	—	+	+	0	0	0	Death following reoperation for stricture
	2	24	0	24	+13	—	—	+	+	0	0	0	Stenosis of valve
	13	50	0	50	+36	—	0 36	0	0	0	0	0	Undetermined
Histamine given (8 animals)	15	60	3	63	+34	—	—	0	0	2	3	3	Sacrificed—Hematemesis
	9	60	12	72	+49	—	—	0	0	3	0	0	Sacrificed—Hematemesis
	17	60	22	82	0 72 0 32 +67	0 32	0 32	0	0	2	2	0	Sacrificed because of esophagogastric margin erosion
	10	60	24	84	+42	+16	—	+	0	2	4	0	Perforated gastric ulcer
	14	61	27	88	+35	+35	0 35	+	0	2	4	0	Perforated gastric ulcer
	7	55	45	100	+44	—	—	+	+	2	2	1	Esophagogastric marginal erosion
	16	60	44	104	0 33	0 33	0 33	0	+	3	4	0	Perforated gastric ulcer
	6	62	45	107	+14	—	—	0	0	2	2	3	Sacrificed

DISCUSSION

The deaths in this series were encountered at the beginning of the study when experience was being gained with the procedure. As experience increased, the technique became simpler and less time-consuming and the results improved accordingly.

It became apparent that the type of valve constructed in group I could be rather easily inverted in retrograde position up the esophagus. This undesirable complication was prevented by the "mesentery" type of esophageal suspension used in group II. Surgical stenosis encountered early was avoided subsequently by distending the esophagus just enough to obliterate the longitudinal folds and making the width of the mucosa-submucosal flap to conform to this circumference.

Esophagoscopic examination revealed spontaneous esophagitis in one of 12 animals (8 per cent). Following chronic histamine stimulation 10 of 12 animals revealed esophagitis of varying severity. While this appears to be an overwhelming incidence, in only one animal did esophageal pathology occur by itself. In fact, 10 of 12 animals also revealed gastric pathology. In six of these 10 cases the gastric pathology was more severe than the esophageal pathology. Five perforated gastric ulcers were observed. In the remaining 4 instances the gastric and esophageal lesions were of equal degree. Duodenal lesions were noted in only 4 animals. The predominance of gastric contrasted with duodenal pathology has been observed in this laboratory in other experiments where the experimental preparation included a portion of the stomach in the intrathoracic position. No explanation can be offered at present for this phenomenon.

We must assume that some protection of the esophagus was afforded by the procedure, for the incidence and severity of the esophagitis was appreciably less than that encountered in animals undergoing a conventional esophagogastric anastomosis accompanied by bilateral vagotomy and a variety of drainage procedures combined with a 50 per cent upper gastrectomy.[4] Nonetheless, the results leave a great deal to be desired. However, certain aspects of this problem deserve further comment. In this study a bilateral vagotomy has been effected which should exert a protective effect. This effect has been negated by the administration of histamine, which acts directly on the parietal cell. Furthermore, an adequate drainage procedure appears to be extremely important in the presence of a vagotomy. A pyloromyotomy was utilized in the study herein reported; however, subsequent work experimentally[5] has indicated that it is an inadequate drainage procedure. Conceivably, additional esophageal protection would be afforded by the substitution of a Finney pyloroplasty for the pyloromyotomy.

SUMMARY

The esophagogastric junction was replaced in 20 dogs by a surgically constructed valve made from the lower esophagus and upper stomach. The valve was competent in approximately half of all animals tested. By esophagoscopy only one of 12 animals revealed spontaneous esophagitis. Under chronic histamine stimulation 10 of 12 dogs revealed esophagitis of varying severity. In only one animal, however, was the esophageal pathology more

stimulation developed esophagitis in from 13 to 45 days. By contrast, of the 4 animals in group II receiving histamine stimulation, 2 (50 per cent) showed esophagitis and 2 of these animals died from perforated gastric ulcers (Fig. 3). The only 2 valves competent to apomorphine-induced vomiting occurred in group II.

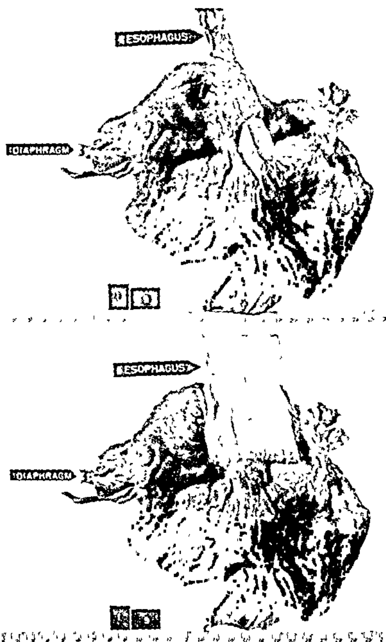


Fig 3 Dog No 19 This animal died 63 days postoperatively of a perforated gastric ulcer after 3 days of histamine stimulation The valve was competent without stenosis and there was no esophagitis. *Above*, Stomach opened to show esophageal nipple with a dural forceps inserted in the stoma. *Below*, Nipple opened to show normal esophageal mucosa. Note perforated gastric ulcer lateral to the esophagus.

tomy seven years previously for carcinoma, without construction of a reservoir, in whom a reservoir was recently constructed.

METHOD

Each patient was placed on a weighed diet with determination of the ingested fat and nitrogen throughout the period of study. Three days after the initiation of the diet in each instance, a carmine marker was administered to determine the beginning, and also to demarcate the end of each period. The feces for each period were homogenized in a Waring Blendor, weighed, and fat and nitrogen content determined on aliquot samples. The fat was determined by a modification of the method described by van de Kamer[1] and nitrogen by the macro-Kjeldahl procedure.

Case 1. B A., GH-42509, a 61 year old white male, had a total gastrectomy performed on November 6, 1953 for what proved to be a benign gastric ulcer. A substitute gastric pouch was constructed for him by the method described by Longmire and Beal[2] as modified by Hays[3] Since the inflammation about the ulcer involved the diaphragm, a portion of this as well
was just below the inf
weighed 130 lbs , a loss
in weight and now weig
arise in the course of this patient, he was selected for study of his fat and nitrogen absorption. An initial study over a single ten day period was made in this patient ten weeks following his gastrectomy. He was again studied for three separate five-day periods eight months later.

Case 2. G E S , GH-4461, a 44 year old white male, was operated upon April 10, 1947 for carcinoma of the stomach with lymph node metastases. He had a total gastrectomy. Intestinal continuity was reestablished by an end to side esophagojejunostomy. A jejunojejunostomy was performed below the first anastomosis. This patient has subsequently had numerous admissions to this hospital because of nutritional difficulties. His weight had been 30 to 40 lbs under normal, and he had been troubled by frequent attacks of diarrhea. It was believed that this patient might also benefit from the formation of an artificial reservoir. Accordingly, after fat and nitrogen intake-excretion were studied for three five-day periods, a jejunal reservoir was constructed just distal to the enteroenterostomy. At operation no evidence of recurrent carcinoma was seen. Four months later, the assimilation studies were repeated over three similar five-day periods.

RESULTS AND DISCUSSION

The data obtained from this study of the intake-excretion of fat and nitrogen following construction of a substitute reservoir in two patients who had had total gastrectomy are given in Tables 1 and 2, respectively.

Table 1. Daily Intake and Excretion of Fat and Nitrogen by Case 1

	1		2		3		4	
PERIOD*	INTAKE	FECAL	INTAKE	FECAL	INTAKE	FECAL	INTAKE	FECAL
Fat, grams	72.0	21.4	77.0	7.35	77.0	8.18	75.2	10.7
Nitrogen, grams	14.17	2.75	13.6	2.22	13.6	1.91	14.11	1.89

* The initial period was for 10 days; the subsequent three periods of study were for 5 days.

There are three possible explanations for the lessened amount of excreted fat in case 1 ten months following surgery from that found shortly after the operation. First, that the intestinal tract, in some way as yet unexplained, has partially compensated for the loss of the stomach by improvement in fat assimilation. A second possibility is that the reservoir has come

severe than the gastric pathology. By the addition of suggested modifications further experimental trial appears warranted.

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THE INFLUENCE OF A SUBSTITUTE GASTRIC RESERVOIR UPON THE ABSORPTION OF FAT AND NITROGEN IN PATIENTS WHO HAVE HAD TOTAL GASTRECTOMY*

JOHN D BRIGGS, JAMES A. HALSTED, AND WILLIAM P. LONGMIRE, JR.

Serious problems are sometimes encountered in the management of patients following total gastrectomy. Nutritional disturbances, particularly inability to gain weight, and anemia have been of concern to surgeons and others interested in the postoperative care of these patients. Many varieties of gastric reservoirs have been constructed in an attempt to overcome these difficulties. Most reports of their use have been favorable in that there is a lessened incidence of the dumping syndrome and esophageal regurgitation and improvement in the nutritional status of those patients

Abnormal amounts of fat and nitrogen are frequently found in the stool of patients who have had total gastric resection, indicating probable decreased absorption of ingested food The physiologic cause for this has not been determined. In addition, there is evidence to show that often oral intake is inadequate even though the patient believes he is eating normal amounts. Whether one or the other, or both of these factors is the major cause of nutritional difficulties has not been established

Since the purpose of a substitute gastric reservoir is to increase intestinal absorption by (1) enabling patient to take increased amount of food without discomfort, (2) prolongation of the transit time and perhaps (3) improved mixing of food with the bile and pancreatic juice, it was believed to be of value to study the absorption of fat and nitrogen in patients who had had this operation performed Accordingly, two patients were selected, one who had had a gastric reservoir constructed following total gastrectomy for what proved to be a benign ulcer, and the second who had had a total gastrec-

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ing amount so that the percentage of fat excreted remained the same, being about 4 per cent. They also pointed out that while a portion of the fecal fat is probably derived from sources other than unabsorbed dietary fat, the major fraction is related to the ingested fat. For this reason, Everson[7] suggests that it is more accurate to compare absolute fecal fat excretion values, rather than percentages, if the diet contains less than 80 grams of fat per day.

The results of nitrogen determination show changes similar to that of fat assimilation. Case 1 excreted more than normal amounts of nitrogen initially, but his average for the three subsequent metabolic periods is at the upper limit of the normal value (2 grams daily). The second patient

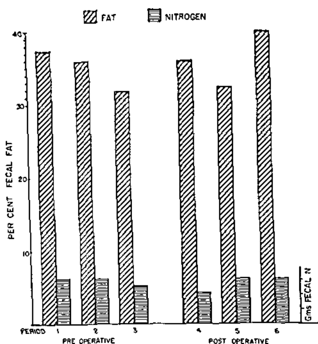


Fig. 2. Graphic representation of fecal fat and nitrogen in case 2 prior to and after construction of the jejunal food pouch.

has had increased nitrogen in his feces, and there has been no significant change since construction of the reservoir.

SUMMARY AND CONCLUSIONS

The fat and nitrogen absorption was studied in two patients who had had construction of a substitute gastric reservoir following total gastrectomy.

The first patient, while initially having increased fecal fat and nitrogen when studied ten months postoperatively, was found to have values at the upper limits of normal. It is postulated that this improvement is due either to unknown factors causing improved absorption of fat and nitrogen, or to improved functioning of the jejunal pouch.

The second patient, who had a gastrectomy for carcinoma seven years ago, had no change in fecal fat and nitrogen four months after construction of the reservoir from that found prior to the operation. The failure in this patient may be due to the unphysiologic intestinal continuity resulting from by-passing the duodenum.

to function as an artificial stomach and ingested fat is liberated more slowly to the intestine, resulting in better absorption. Emery[4] found that there was improved utilization of fat in gastrectomized dogs when it was given in small amounts. The third possibility, that of normal variability in absorption, seems unlikely in view of the steady gain in weight of this patient

The construction of the reservoir in case 2 has not improved his nutritional status except he believes that he is able to eat a greater quantity at each meal. His diarrhea has continued unabated and undoubtedly contributes to the excessive amount of fecal fat. It may be that improvement in his nutrition would result if his intestinal continuity were reestablished through the duodenum as in case 1.

Table 2. Daily Intake and Excretion of Fat and Nitrogen by Case 2

	1		2		3	
PERIOD* (PREOPERATIVE)	INTAKE	FECAL	INTAKE	FECAL	INTAKE	FECAL
Fat	94 3	35 2	94 3	34 0	96 2	30 7
Nitrogen	12 96	3 19	12 96	3 21	12.78	2 61
PERIOD* (POSTOPERATIVE)	4		5		6	
Fat	108 0	38 8	108 0	40 5	90 9	36.2
Nitrogen	12 01	2 15	12 01	3 04	12.37	3 15

* Each period of study was for 5 days

Wollaeger and his co-workers[5, 6] found that normal individuals excrete 1.8 to 6 7 grams of fat daily on a diet containing 101.6 grams When the amount of fat in the diet was doubled, the fecal fat increased a correspond-

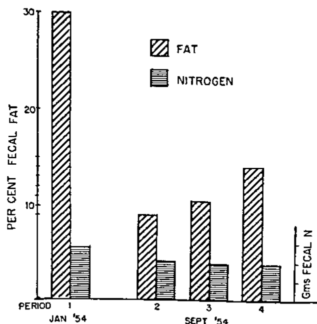


Fig 1. Graphic representation of fecal fat and nitrogen in case 1, ten weeks and ten months following total gastrectomy.

To understand the experiments to be described, it should be recalled that the diaphragm of the dog contains both left and right crura which together form the boundaries of the esophageal hiatus. The right crus is an extremely thin band of muscle fibers which forms the right and posterolateral boundary of the hiatus. From its lumbar origin it courses cephalad and dorsad from right to left to join the left crus behind the esophagus. At operation, the contractions of the right crus seem feeble, without force and with little if any purpose.

The left crus is a thick band of muscle fibers which forms the left lateral and anterior boundaries of the esophageal hiatus. It courses cephalad from its lumbar vertebral origin, swings ventrad around the left side of the esophagus and thence comes to lie in the angle formed by the junction of the left lateral wall of the esophagus with the cardia of the stomach. When this crus contracts during inspiration, it not only compresses the walls of the esophagus but it increases the angulation between its left lateral wall and [illegible] same as that of the right crus in the human being, which splits to form the esophageal hiatus and passes the esophagus at its junction with the gastric cardia.

PROCEDURE

In our experiments, four operative procedures were performed on normal adult dogs. Three of these procedures were designed to weaken or destroy any function by which the diaphragm might prevent regurgitation of stomach contents into the esophagus, and the fourth served to create a permanent partially thoracic stomach. Each procedure was performed on a different group of dogs. A left thoracotomy incision, and general anesthesia produced by the intravenous administration of pentobarbital sodium solution, were used in each instance.

In a group of 10 dogs we excised the left crus of the diaphragm. The procedure consisted of identifying the crus, dissecting it free from the underlying phreno-esophageal ligament, and excising it close to its origin and insertion Usually, we could remove a strip of muscle about 1.5 cm. in width and 7 to 10 cm. in length. After the excision, the gastric cardia could be seen bulging up through the hiatus above the left leaf of the diaphragm into the thoracic cavity, but separated from the thoracic cavity by the semitransparent peritoneum and diaphragmatic fascia.

In a group of 4 dogs we excised the right crus of the diaphragm. The technique employed was similar to that used for excision of the left crus. However, it was also necessary to incise and later close a segment of the posterior mediastinal pleura to approach the right diaphragmatic crus.

In another group of 4 dogs we excised both crura.

In a group of 9 dogs we created a permanent partially thoracic stomach by dissecting free the phreno-esophageal ligaments which anchored the lower end of the esophagus to the esophageal hiatus. The upper one-third of the mobilized stomach was then drawn through the hiatus into the thoracic cavity and in that position was reattached to the margins of the reconstructed crura that formed the hiatus.

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CANINE ESOPHAGITIS FOLLOWING EXPERIMENTALLY PRODUCED ESOPHAGEAL HIATAL HERNIA*

VINCENT J. GIUSEFFI, JR., JOHN H. GRINDLAY, AND HERBERT W. SCHMIDT

The regurgitant esophagitis that is associated with esophageal hiatal hernia has long been observed However, the mechanisms responsible for this entity have not been satisfactorily explained.

It is generally accepted that esophagitis may develop when gastric acid chyme bathes a nonresistant esophageal mucosa. Such a condition prevails when competency of the gastric cardia is disrupted Normally, such competency is thought to be maintained by the individual or composite actions of three functioning mechanisms· (1) the cardiac valve, (2) the cardiac sphincter and (3) the diaphragmatic pinchcock mechanism The relative importance of each is open to question.

The loss of sphincter and valve actions in that condition in which a congenital short esophagus with partially thoracic stomach exists has been incriminated as the cause of incompetency of the gastric cardia.[1] Also, the combination of an ineffectual intrinsic sphincter and a poorly functioning diaphragmatic pinchcock mechanism, frequently present in the sliding type of esophageal hiatal hernia, has been pointed out as the cause of cardiac incompetency.[2] In the experiments to be reported, we have attempted to selectively eliminate diaphragmatic action as a mechanism of maintaining competency of the gastric cardia.

* [illegible]

twice a day; a cereal meal and a meat meal have been used. No other method of increasing gastric secretion has been employed.

At the time of this writing the experimental work has not been concluded —approximately one-third of the dogs are still under observation. Thus far, however, esophagitis has developed in the 9 dogs (in the group of 10) in which a sliding type of hiatal hernia was produced by excision of the left crus of the diaphragm; the only animal that did not have subsequent esophagitis was the dog, previously discussed, in which a hiatal hernia did not develop after operation. Generally, the severer forms of esophagitis developed in those dogs which had the greater degrees of cardiac incompetency and regurgitation as determined fluoroscopically. The esophagitis was mild in 1 dog; it healed and later recurred. The esophagitis was moderately severe in 3 dogs, in 1 of which it recurred after initial healing. The esophagitis was severe in 5 dogs, in all of which it subsequently healed and recurred.

Usually 8 to 16 weeks elapsed before any inflammation of the esophagus was evident. After the onset, the inflammation increased to its greatest severity in about 6 weeks, and in another 6 weeks healing would become evident. In those 7 dogs in which chronic recurrent ulcerative esophagitis developed, the second episode of esophagitis was initiated anywhere from 4 to 26 weeks after the original inflammation had completely subsided. Clinically, the 5 dogs with severe esophagitis had signs of dysphagia, and regurgitated or vomited after ingestion of food during their initial illness. At that time an associated loss of weight was obvious. The dogs with moderate or mild reactions lost weight at similar times but remained asymptomatic.

In the group of 4 dogs that underwent excision of the right crus, hiatal hernia did not develop, as previously mentioned, nor was cardiac incompetency demonstrable by fluoroscopy. However, 1 of these dogs had a mild esophagitis which healed and did not recur. On several occasions this particular dog presented minimal incompetency of the gastric cardia at esophagoscopy. In the remaining 3 dogs of this group esophagitis did not develop at any time. Necropsy confirmed the absence of hiatal hernia in all 4 dogs. Actually, in this group we did not expect regurgitant esophagitis to occur. We do not believe that the right crus of the dog's diaphragm is of importance in maintaining competency of the gastric cardia, from an anatomic or physiologic point of view.

Of the group of 4 dogs which underwent excision of both crura, esophagitis developed in all. In 2 of these the esophagitis was mild and did not recur. In the other 2 dogs the esophagitis was moderately severe; in 1 of these, recurrence was evident 20 weeks after initial healing, and in the other no recurrence was noted. Whereas a greater degree of cardiac incompetency and subsequent esophagitis would be suspected in this particular group, such did not occur. At necropsy, dense adhesions about the posterior and lateral [illegible] ion that the magnitude [illegible] the formation of adhe- [illegible] tal relaxation that may have been induced by the simultaneous excision of both diaphragmatic crura.

Of the group of 9 dogs in which a permanent partially thoracic stomach was created, esophagitis developed in 5. In 1 of these the esophagitis was

Postoperatively, roentgenoscopic examination after the administration of a barium meal was performed to determine the presence or absence of

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incompetency that was associated with the esophageal hiatal hernia if it was present

Also during the postoperative period, esophagoscopy was performed under general anesthesia at intervals of 4 weeks to determine the presence of esophagitis, gastritis of the superior portion of the stomach, or possible esophageal shortening. The degree of incompetency of the gastric cardia and the magnitude of regurgitation could also be estimated endoscopically. Periodically during examination a Kodachrome motion picture was made of each dog with the Holinger-Brubaker camera.

We classified esophagitis on the basis of gross findings at endoscopic examination, as follows Mild esophagitis is described as a diffuse reaction in which there are hyperemia, edema and congestion of the esophageal mucosa, but in which the mucosal layer remains intact. Moderate esophagitis is defined as localized intense inflammation characterized by mucosal erosion or epithelial desquamation Severe esophagitis implies frank ulceration of the

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within the longitudinal folds of the esophageal mucosa. Specimens are now being taken at necropsy. These are being sectioned and studied microscopically. Our classification will ultimately be based on both the gross and microscopic findings.

RESULTS

Esophageal Hiatal Hernia. In 9 of the 10 dogs which underwent excision of the left crus, roentgenoscopic examination revealed an esophageal hiatal hernia. Postmortem examination of the 1 dog in which such a hernia failed to develop postoperatively revealed dense adhesions about the hiatus; the edge of the left half of the diaphragm adjacent to the site of excision of the crus was markedly bound down to the gastric cardia along the greater curvature. Hiatal hernia had not occurred through these dense adhesions. Regurgitation was demonstrable in all of the dogs in which hiatal hernia had developed.

The group consisting of 4 dogs which had undergone excision of the right crus uniformly presented no evidence of hiatal hernia, nor could regurgitation be demonstrated fluoroscopically in any case.

Of the 4 dogs which had undergone excision of both crura, hiatal hernia was present in all, but regurgitation was evident in only 2 of these

Roentgenoscopic examination confirmed the presence of the partially thoracic stomach in each of the 9 dogs in which such a condition had been created. However, the degree of regurgitation associated with the hiatal hernia varied widely. In 5 of the 9 dogs no regurgitation could be demonstrated by manual compression of the animal's abdomen, but in the remaining 4 dogs regurgitation could be demonstrated

Esophagitis. So far, the animals have been followed postoperatively for periods ranging from 12 to 18 months, during which they have been fed

2. The left crus of the dog's diaphragm is mainly responsible for the diaphragmatic pinchcock mechanism in the dog.

3. The esophagitis associated with hiatal hernia is usually of a recurrent nature and characteristically undergoes remissions.

Although we have not yet completed our studies, we wonder whether we have not created in dogs in which the left crus of the diaphragm was excised the factors which produce an ascending fibrosis of the esophageal wall. We intend to study the remaining dogs of this group for a longer period of time in order to learn whether further changes will occur, such as shortening of the esophagus.

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EXPERIENCES WITH THE INTERPOSED JEJUNAL SEGMENT OPERATION COMBINED WITH ADJUNCT PROCEDURES IN THE PREVENTION OF ESOPHAGITIS*

An Experimental Study

DAVID H. DILLARD AND K. ALVIN MERENDINO

Reflux esophagitis constitutes an important problem from the viewpoint of surgical therapy.

Previous reports from this laboratory indicate that whenever the esophagogastric sphincter is excised together with a bilateral vagotomy and restoration of gastro-intestinal continuity is effected by esophagogastrostomy, esophagitis is a constant sequela. This is observed even when the procedure is accompanied by an extensive upper gastrectomy.[1] These observations stress again the extreme sensitivity of the esophagus to acid and pepsin. In searching for an intestinal segment more resistant than the esophagus to acid-peptic digestion, the jejunum was considered a satisfactory tissue for interposition between the esophagus and the stomach, hoping thereby to isolate the esophagus from contact with gastric secretion. The choice of jejunum was not accidental, for earlier experiments indicated that even down to mid-jejunum no important increased inherent sensitivity of this mucosa to acid-peptic digestion existed[2-4]

However, mindful of the hesitance of others in effecting jejunogastric anastomoses, a pilot study was outlined in order to determine if ulcerative disease would occur in the lower portion of the interposed jejunum and to

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mild and recurred after initial healing. In 2 dogs the esophagitis was moderately severe, it recurred in 1 of these but not in the other. The esophagitis was severe in 2 dogs, but in only 1 of these did it subsequently recur. Moreover, an associated chronic gastritis developed in 6 of these dogs The involved area was situated immediately below the esophagogastric junction adjacent to an area of esophagitis. The gastritis occurred concomitantly with severe esophagitis in 2 dogs and with moderate esophagitis in 2 dogs. In the other 2 dogs the gastritis was marked but it was not associated with regurgitant esophagitis at any time.

The esophagitis that developed after creation of a partially thoracic stomach did not bear any direct relationship to the degree of cardiac incompetency noted at fluoroscopic examination. Usually the esophagitis in this group of dogs began about the fourth postoperative week, reached its peak in the tenth week, and appeared completely healed by the twenty-first week. In the 2 dogs in which the degree of esophagitis was severe, 22 to 25 weeks elasped before any inflammatory changes developed. Recurrent esophagitis, when noted in 3 of the dogs, appeared to have its onset 4 to 27 weeks after initial healing had occurred. Clinically, in 3 dogs of this group signs of dysphagia, regurgitation or emesis developed after ingestion of food; each had marked incompetency of the cardia on fluoroscopic examination and each had gastritis postoperatively. The gastritis was associated with esophagitis in 2 instances, in 1 of these the esophagitis was severe and in the other it was of moderate degree. Loss of weight was never conspicuous in any animal of this group.

SUMMARY AND CONCLUSIONS

Esophageal hiatal hernia developed after excision of the left crus of the diaphragm in 9 of 10 dogs. Subsequently, all 9 dogs had regurgitant esophagitis, and 7 of the 9 had chronic recurrent ulcerative esophagitis.

Esophageal hiatal hernia was not produced by excision of the right crus of the dog's diaphragm nor did regurgitant esophagitis occur readily after such excision In such a group of 4 dogs, 1 subsequently had mild esophagitis which did not recur.

Excision of both crura of the diaphragm in 4 dogs resulted in the formation of esophageal hiatal hernia in all 4, and esophagitis also developed

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that seen in dogs in which only the left crus was excised. This was thought to be due to the formation of adhesions at the locus of operation, the adhesions partially negating hiatal relaxation, hiatal herniation and subsequent incompetency of the gastric cardia.

Regurgitant esophagitis developed in 5 of 9 dogs in which a partially thoracic stomach was created. The esophagitis attained chronicity and recurred in 3 of the 5, but its severity did not approach that observed in dogs

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stomach alone is created.

2 animals in the entire series of 15 dogs exhibited slight microscopic esophageal inflammation. One of these occurred in an animal (Y-75) with the shortest interposed segment (4.5 cm.). This area was immobilized by scar tissue which possibly adversely affected its peristaltic activity.

In order to explain esophageal protection by the interposed jejunal segment, one must entertain the possibility that such a segment behaves as a physiologic sphincter. This isoperistaltic segment retains its peristaltic pattern, which undoubtedly prevents contact of the gastric chyme with esophageal mucosa. In several animals at the time of sacrifice, vigorous peristalsis was observed in the interposed jejunum. In others, this was noted by means of barium studies. Furthermore, in these experiments not only was

Fig. 1

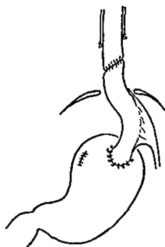
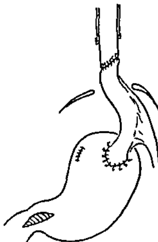


Fig. 2



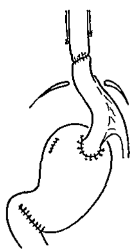


Fig. 3



Fig. 4

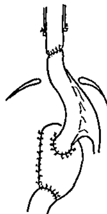


Fig. 5

Fig. 1. Group I: Basic preparation only.
Fig. 2. Group II: Basic preparation plus Rammstedt pyloromyotomy.
Fig. 3. Group III· Basic preparation plus Finney pyloroplasty.
Fig. 4. Group IV. Basic preparation plus antrumectomy.
Fig. 5 Group V. Basic preparation plus 75% lower gastrectomy.

evaluate adjunct procedures which might conceivably be of value should this complication ensue.

Our experiences in this regard constitute the basis of this report.

METHOD

Seventeen adult mongrel dogs were used in this study. Anesthesia consisted of intravenous Nembutal supplemented with intermittent endotracheal ether and oxygen administered by means of a Palmer respirator. Either an abdominal or a left thoraco-abdominal incision was used. Transection of the esophagus with excision of the esophagogastric junction, closure of the cardia, bilateral supradiaphragmatic vagotomy and interposition of an isoperistaltic segment of jejunum between the esophagus and a neostoma on the anterior surface of the fundus of the stomach were performed on each animal. In addition to evaluating the basic preparation outlined, various adjunct procedures were incorporated in order to determine their effect. Consequently, 5 groups of animals were developed as follows (see Table 1 and Figs. 1-5).

Group 1. The above basic preparation only (3 dogs).

Group 2 Basic preparation [illegible] logs).

Group 3. Basic preparation [illegible]

Group 4 Basic preparation [illegible]

Group 5. Basic preparation plus conventional 75 per cent lower gastrectomy (3 dogs).

Postoperatively, these animals were given 300,000 units of penicillin I.M., q.d. for 3 days. Clysis were administered for 3 to 6 days depending upon the general condition of the animal. Fluids were allowed by mouth on the fourth or fifth day. A special diet of Osterized milk and Friskies Dog Food was introduced and gradually increased as tolerated over the subsequent week.

After a 30 day recovery period all animals were begun on daily injections of 30 mg. of histamine base in oil and beeswax Histamine phosphate was employed in this preparation and each new mixture was tested for potency on Heidenhain pouch dogs and also concurrently was given to a control series of animals Each preparation was found to be adequately ulcerogenic. Following a course of 45 days on histamine, all survivors were sacrificed Gross abnormalities were noted and representative photographs were taken Histologic sections were taken routinely as follows. through the esophagus, 5 cm. proximal to the esophagojejunal anastomosis, across the esophagojejunal anastomosis, from the center of the jejunal segment and across the jejunogastric anastomosis Other sections were taken as indicated.

RESULTS AND DISCUSSION

An inspection of the results recorded in Table 1 reveals many interesting aspects which deserve comment First of all, it should be pointed out that under the conditions of these experiments a bilateral vagotomy has been effected plus esophageal transection One might, with confidence, anticipate that some protection against acid-peptic ulceration would be afforded by this maneuver. However, by the administration of histamine, which acts directly on the parietal cell, the protective effect of vagotomy has been by-passed. In spite of severe histamine administration, gross esophagitis was not observed in a single animal, regardless of the operation performed Only

the incidence of jejunal pathology lower than anticipated, but jejunal reaction of even the most minimal sort was never encountered more than 14 cm. proximal to the jejunogastrostomy. These findings suggest that the retained peristaltic pattern is self-protective for the jejunal segment as well. However, one must consider the potential protective action of salivary juice, which is ever present.

An inspection of the data indicates that only in groups I and II was any gross or microscopic jejunal pathology observed. The combination of gastric atony and pylorospasm associated with vagotomy, plus the presence of an intact stomach subjected to histamine stimulation (group I) constitutes the

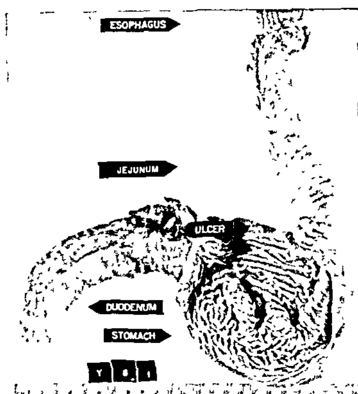


Fig. 6 Dog V-81 (group IV). Died of perforated duodenal ulcer following 6 days of histamine stimulation. No jejunitis or esophagitis seen grossly or by microscopic examination.

severest test of all. The similarity of the results with regard to jejunal involvement in groups I and II suggests that a Rammstedt pyloromyotomy is an inadequate drainage procedure. In short, groups I and II represent essentially the same experimental preparation. It is not surprising that the incidence and severity of gastric and duodenal pathology was highest in these two groups.

By contrast, when a Finney pyloroplasty (group III), an antrumectomy (group IV) or a conventional 75 per cent subtotal gastrectomy (group V) was added to the basic procedure no pathology occurred in the interposed jejunal segment. However, the antrumectomy series (group IV) appeared to be associated with severe duodenal complications (Fig. 6).

These groups are too small to be of statistical significance but observations

Table 1. Summary of Procedures and Results

OPERATIVE PROCEDURE	DOG NO.	SURVIVAL DAYS: PRIOR HISTORY	SURVIVAL DAYS: HISTORY	SURVIVAL DAYS: TOTAL	LENGTH OF JEJUNAL SEG IN CM	GROSS AND MICROSCOPIC PATHOLOGY*: ESOPHAGUS	JEJUNUM	STOMACH	DUODENUM	COMMENTS
I. Basic preparation only	Y-70	43	6	49	18	0	1	0	4	Perforated duodenal ulcer
	Y-73	40	45	85	13	0	0	0	0	Sacrificed
	Y-82	30	21	51	8	0	3	0	2	Esophagogastric anastomosis and diaphragm bound together with adhesions
II. Basic preparation plus Rammstedt pyloromyotomy	Y-74	30	8	38	12	0	0	0	4	Perforated duodenal ulcer
	Y-75	30	5	35	4 5	1	2	0	4	Short jejunal segment immobilized in scar tissue
	Y-90	30	39	69	19	0	4	2	3	Perforated jejunal ulcer
III Basic preparation plus Finney pyloroplasty	Y-92	30	44	74	22 5	0	0	0	0	Sacrificed
	Y-123	30	45	75	16	0	0	0	0	Sacrificed
	Y-125	30	45	75	14	0	0	2	0	Sacrificed
IV. Basic preparation plus antrumectomy	Y-80	30	4	34	12	1	0	1	4	Perforated duodenal ulcer
	Y-81	30	6	36	19	0	0	0	4	Perforated duodenal ulcer
	Y-84	47	46	93	11.5	0	0	0	0	Sacrificed
	Y-177	30	33	63	21	0	0	4	4	Perforated duodenal ulcer
	Y-180	31	4	35	12	0	0	0	4	Perforated duodenal ulcer
V. Basic preparation plus 75% lower gastrectomy	Y-109	30	45	75	9	0	0	0	0	Sacrificed
	Y-164	58	47	105	11 5	0	0	0	0	Sacrificed
	Y-167	59	6	65	24	0	0	0	0	Died of hair bezoar ileus

* The pathologic findings are graded according to gross and microscopic findings as follows: 0, normal without inflammation; 1, microscopic inflammation; 2, erosions not greater than 1 cm in diameter and not extending beyond the submucosa, 3, erosions greater than 1 cm. in diameter or ulcers extending into the muscularis, 4, perforated ulcer

phagogastric junction, certain undesirable secondary effects attributable to peptic acid regurgitation and esophagitis make their appearance. Following this, any of the generally known complications of peptic ulceration may occur. Previously, Kiriluk and Merendino[2] have shown a protective effect in preventing esophageal complications by the use of a "fish mouth" valve-like esophagogastrostomy. The concept was formulated that a procedure could be performed on the distal esophagus which would more nearly

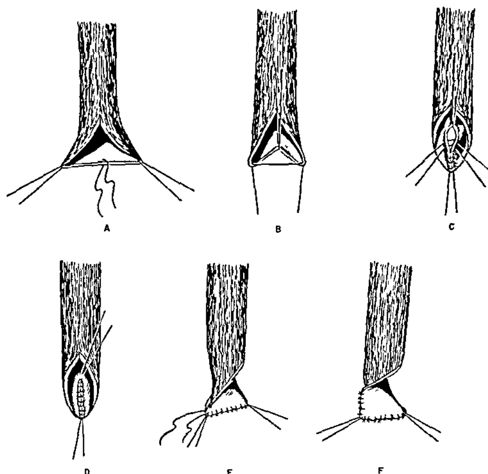


Fig. 1. *A*, The anterior wall of the esophagus is split longitudinally for a distance of 2 to 3 cm. *B*, A guide suture is placed through the midpoint of the posterior esophageal wall opposite the origin of the anterior wall incision, and interrupted sutures elevate the midportion of the posterior wall (*C*). *D*, The suture line on the posterior esophageal wall has been completed. On placement of these interrupted sutures, the esophageal mucosa of the entire circumference is everted (*E*), and this everted mucosa is approximated in turn by interrupted sutures (*F*), forming a quadrangular flap.

approximate the functions of the normal "hiatal valve" than a simple end-to-side or end-to-end esophagogastrostomy

Healthy, adult mongrel dogs were used. The esophagus was transected 1 cm. above its inosculation into the stomach and the cardia was transected about 1 cm. below. This wound was then closed. In 6 animals the vagal nerves were preserved; in 8 others both vagi were transected. Esophagogastrostomy was carried out in each series with formation of a valve. The technique used is delineated in the accompanying illustrations (Figs. 1-3).

from this pilot study seem worth additional comment. In those animals (groups III, IV, V) where the adjunct procedure results in a large efferent channel from the stomach, jejunal pathology is non-existent. This is so even *in the presence of an intact stomach. Mild esophagitis was encountered in* only 1 of 9 dogs. In these groups the jejunum appears to have greater resistance to acid-peptic digestion than either the stomach or the duodenum

In groups I and II the jejunal segment has greater resistance than the duodenum but appears to be more sensitive than the stomach.

SUMMARY

An experimental preparation was created in 17 dogs consisting of excision of the esophagogastric junction and its replacement by an interposed isoperistaltic segment of jejunum. Each animal had, in addition, a bilateral vagotomy Various adjunct procedures were employed. Under histamine stimulation the interposed jejunal segment, when accompanied by an adequate efferent stoma, even in the presence of an intact stomach, was more resistant to acid-peptic digestion than either the stomach or duodenum. A short jejunal segment in most instances prevented even microscopic esophagitis. The interposed jejunal segment appeared to behave as a physiologic sphincter.

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VALVULAR ESOPHAGOGASTROSTOMY*

A Method of Preventing Peptic Esophagitis Following Esophagogastric Anastomosis

DAVID H. WATKINS, ARTHUR E. PREVEDEL, AND GORDON A. MUNRO

Between the esophagus and the stomach are a group of structures known collectively as the cardiac mechanism whose purpose is to insure the aboral transit of food. The details of this mechanism have been described previously.[1]

If this cardiac mechanism becomes disturbed by a pathologic development, such as an esophageal hiatal hernia, or by surgical means such as cardioplasty or esophagogastrostomy for obstructive lesions at the eso-

* From the Department of Surgery, University of Colorado School of Medicine, Denver This work was supported by a grant from the V. F. Taylor Foundation.

EXPERIMENTAL RESULTS

Six dogs were prepared in which the vagi were preserved. Although there was a transient period in the early postoperative course when a mild

Fig. 4.



Fig 5.

Fig 4 This specimen shows the end result of the healing process with the inosculation of the esophagus into the stomach. There is no evidence of esophagitis. The mushroom projection of the valve is seen (Reproduced by permission of the Journal of Thoracic Surgery, Vol. 28.)

Fig 5. When the edges of the cut esophagus are approximated the valve is seen to almost completely occlude the stoma. (Reproduced by permission of the Journal of Thoracic Surgery, Vol. 28.)

In each case the esophagogastrostomy lay above the level of the diaphragm in the posterior mediastinum. The end result of the procedure following healing is demonstrated in Figs. 4 and 5.

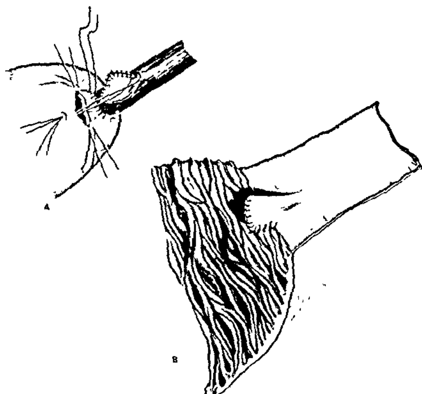


Fig. 2 The method of performing the end-to-side esophagogastrostomy is shown. The posterior seromuscular layer has been placed The valve is drawn into the stomach prior to completion of the anterior rows of sutures The position of the valve following completion of the anastomosis is shown in B.

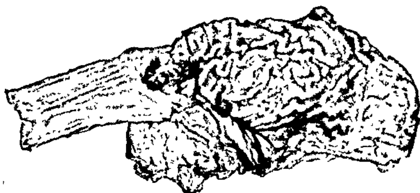


Fig. 3. In the axially sectioned specimen from a freshly completed procedure the relationship of the valve to the lumen of the esophagus and stomach is shown.

CONCLUSIONS

1. Valvular esophagogastrostomies were carried out in dogs in which the entire stomach pouch was preserved.
2. With both vagi transected or preserved, these dogs have survived without evidences of esophagitis for periods varying from 30 to 395 days.
3. This procedure has been carried out in human patients with encouraging clinical and roentgenologic results. It has the advantage of being applicable to both high and low esophagogastrostomies.

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STRANGULATION OBSTRUCTION*†

Postoperative Antibiotic Protection

ISIDORE COHN, JR.

Securing unlimited survival in strangulation obstruction by the proper combination of preoperative bowel sterilization and postoperative antibiotics[1] stimulated interest in the results that might be obtained without preoperative preparation. The lack of clinical application of preoperative therapy made evaluation of postoperative therapy alone seem worthwhile. It was therefore decided to study experimental strangulation obstruction with antibacterial therapy limited to the postoperative period.

TECHNIQUES

Dogs were vaccinated against distemper, and were kept in the animal colony a minimum of four weeks. The dogs were observed for six days preoperatively, during which time they were allowed water and dog biscuits No preoperative medication was used Food was withheld for 18 hours prior to surgery and water four hours. Operative procedures utilized sterile technique and intravenous Nembutal anesthesia. A plastic tube inserted in the femoral vein provided for parenteral therapy during the period of observation.

The abdomen was opened through a midline incision and the omentum removed. The small bowel was divided 75 per cent of the distance from the ligament of Treitz to the cecum. After a culture was taken from the

* From the Department of Surgery, Louisiana State University School of Medicine, New Orleans The investigation was supported in part by a research grant E-524 from the National Microbiological Institute of the National Institutes of Health, Public Health Service.

† Achromycin used in this study was [illegible] the American Cyanamid [illegible] ies Division of [illegible] butal were supplied by [illegible] llin, and Nembutal [illegible] Baxter Laboratories. Amigen and 50[illegible] Johnson Co.

esophagitis was present in most, as indicated by slight erythema, these animals have survived from 118 to 395 days with no evidence of esophagitis or its sequelae.

Fourteen dogs were similarly prepared with both vagi transected. Six animals died in the early postoperative period of gastric dilatation, perforated gastric ulcer and malnutrition. The 8 survival animals have lived for periods from 30 to 75 days without evidence of esophagitis and despite persistent vomiting after feeding.

In all cases a barium meal passed easily through the "hiatal valve" into the thoracic portion of the stomach. With the animal held at 90 degrees, head downward, a small amount of barium regurgitated into the esophagus. However, the main mass of barium was retarded from passing backwards into the esophagus by a marked contraction of the diaphragm which we have termed "diaphragmatic impedance."

CLINICAL APPLICATION

Because of the encouraging results derived from the animal experiments, essentially the same type of valvular esophagogastrostomy was carried out in the following clinical cases.

Case I. E F M, a 68 year old white woman, had an obstructing adenocarcinoma of the stomach extending 2 5 cm above the cardia A large, very fixed, hard mass involving the proximal four-fifths of the stomach was found Resection of the lower third of the esophagus and the proximal two-thirds of the stomach was accomplished A valvular esophagogastrostomy of the type described was carried out, implanting the esophagus into the distal gastric pouch Her postoperative course was very satisfactory. She was able to swallow very well and could take solid food with no difficulty She had no further difficulty with regurgitation or dysphagia Radiographically a nicely functioning valvular anastomosis with an artificial hiatus hernia was demonstrated Repeated esophagoscopies

hemithorax and valvular esophagogastrostomy was then carried out.

The applicability of this procedure is illustrated by these two cases. In the first case the lesion was situated in the cardiac portion of the stomach and in the lower third of the esophagus. It is in this group of cases that esophagogastric anastomosis (esophagogastrostomy, cardioplasty, and so on) is associated with so many poor end-results.

In the second case the lesion was situated in the midportion of the esophagus, and anastomosis was required above the level of the aortic arch The procedure of valvular esophagogastrostomy was easily carried out in this case.

DISCUSSION

Our procedure imitates the two components of the normal "hiatal valve": the oblique inosculation of the esophagus into the stomach, and the difference in intraluminal pressures of the two viscera which approximates the mucosal lips of the cardia.

The efficiency of our valvular anastomosis has been demonstrated by repeated observations of our experimental animals and patients Further, the ease and applicability of this procedure to both high and low esophagogastrostomy is of considerable advantage.

hemolyzed, and odorless throughout these experiments, as in experiments with combined pre- and postoperative antibiotics.[1] This was in contrast to its decided change in character in experiments without antibiotics.[5]

Bacteriology. The bowel lumen was cultured at both operations. Aerobic and anaerobic determinations were made on all specimens (Fig. 1). *Clostridium welchii* and streptococci were found in all animals at the first operation, and *E. coli* and staphylococci were each found in three experiments. An occasional other organism was also found. At reoperation, *Clostridium welchii* was found in only one experiment, while streptococci, *E. coli,* and yeasts were found more frequently.

Peritoneal fluids obtained at about four hours, 50 hours, and 90 to 100 hours from each experiment were subjected to aerobic and anaerobic bacteriologic analysis. In three experiments all cultures were entirely negative, while the fourth one showed staphylococci, *E. coli,* and diphtheroids.

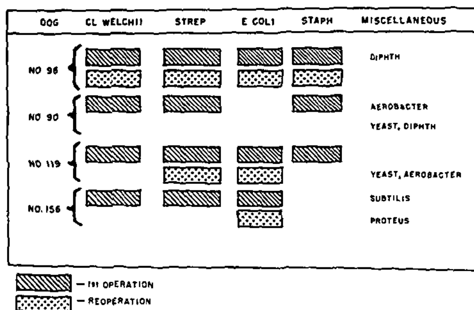


Fig 1. Bacteriology of the bowel lumen at primary operation and reoperation.

In contrast to this were the results in experiments without antibiotics,[5] where the bacterial content of the bowel lumen and peritoneal fluid were similar.

White Cell Counts. The white cell count of the blood rose to a peak at approximately 24 hours It then dropped slightly and began to rise again prior to reoperation (Fig. 2). This resembled curves in the series with pre- and postoperative antibiotics[1] and with postoperative intravenous Aureomycin.[2,3] It is markedly different from that observed in a non-antibiotic series.[4]

The peritoneal fluid white cell count rose in the first 24 to 48 hours, and varied only slightly thereafter (Fig. 2). There are significant differences between this curve and those in all previous series, which we believe can be explained by the bacterial content of the peritoneal fluid.

Fluid Balance. Fluid requirement comparisons were made on the basis of milliliters per kilogram per 24 hours, since this reduced all experiments

lumen, the two ends were closed with Parker-Kerr sutures. A 30 cm. portion of the proximal bowel, 5 cm. or more distant from the closed end, was strangulated by dividing and ligating all veins to this segment, including those vessels parallel to the bowel at each end of the segment. A plastic tube, introduced into viable bowel and threaded into the strangulated segment, was brought out the abdominal wall through a stab wound. Latex tubes placed in the lateral peritoneal gutters and brought out through stab wounds permitted postoperative aspiration of peritoneal fluid.

Electrolytes, dextran, or blood were given during operative and postoperative periods as indicated. Intake-output studies were conducted in metabolism cages.

Antibiotic therapy commenced at the time of strangulation, and continued until reoperation. One gram of Achromycin was introduced into the strangulated bowel at operation, and subsequently Achromycin was dissolved in sterile saline and injected through the plastic tube according to the schedule in Table 1. Penicillin was given intramuscularly 600,000 u. twice daily.

Table 1. Postoperative Antibiotics

Achromycin into strangulated segment	500 mg. q 4 h, 48 hrs, then 500 mg. q. 6 h, until reoperation
Penicillin	600,000 u. I. M., b. i. d.

RESULTS

Four dogs were subjected to this experiment. All survived to be reoperated at 100 hours, when the strangulated segment was resected and intestinal continuity restored. The 100 hour interval between operations was chosen because few other observers have been able to maintain a dog with strangulation obstruction even this long, and because previous studies have shown the bowel has already begun to return to normal by this time.[1] Study of the bowel removed at reoperation further confirmed the superfluous nature of further observation.

Clinical. The dogs responded from anesthesia in 6 to 12 hours, and after that walked about the cage in apparently good condition until the time of reoperation. Vomiting (3 to 6 episodes in different animals) was neither so frequent nor so copious as in experiments without antibiotics.[5] Hematemesis occurred only once in each of three experiments, and a total of four times in the remaining one. Hematemesis was less frequent than in experiments without antibiotics, but slightly more frequent than in experiments where antibiotics were used prior to surgery.[1]

Weight loss, which was a severe problem in a previous series,[1] was not so marked here. We attributed this partly to the use of an invert sugar rather than simple dextrose, since this was the only difference nutritionally from the previous experiments. Intravenous protein, used in both series, was not enough to maintain weight under the severe stress of these experiments.

Peritoneal Fluid. The peritoneal fluid remained pink, coagulable, non-

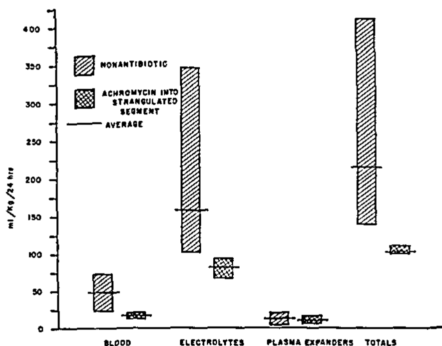


Fig. 3.

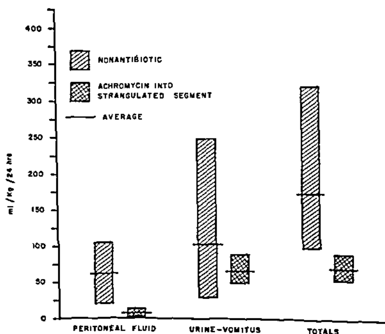


Fig 4

Figs. 3 and 4. Fluid balance comparison of present experiments and non-antibiotic series. Height of bars indicates range of values. Line indicates average value.

to a common denominator (Figs. 3 and 4). The adequacy of therapy was checked by periodic hematocrit and plasma protein determinations. Comparison with intakes in the earlier series without antibiotics[5] showed the significantly smaller requirements for blood, electrolytes, and total intake in this series The smaller blood requirements seem to be the most important difference between this and the previous series. The lesser requirements for blood were no doubt related to the diminished frequency of hematemesis, an
of the
ments.

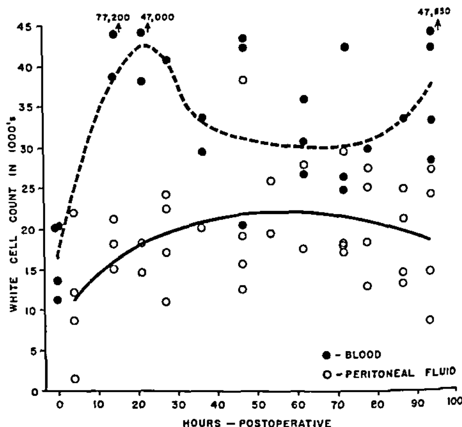


Fig 2 Scatter-graph of white cell count of the blood and peritoneal fluid. Lines represent average values.

closely correlated with those reported in the similar series with both pre- and postoperative antibiotics.[1]

The figures for output were equally impressive when compared with those for the series not receiving antibiotics.[5] The average peritoneal fluid output here was only 5 24, as compared with an average output of 60.3 ml. per kilogram per 24 hours in the non-antibiotic group. Even the urine-vomitus output was only 60 per cent of that in the previous series. The *range* of total output for the two series shows even greater difference than the average of the two series.

Strangulated Segment. The appearance of the strangulated segment was one of the most striking features of the entire experimental work. The

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death in strangulation obstruction: an experimental study. I. Clinical course, chemical, bacteriologic and spectrophotometric studies. Ann. Surg., *130*.857–873, 1949.

EVALUATION OF INTESTINAL ABSORPTION AFTER TOTAL GASTRECTOMY WITH DIFFERENT METHODS OF RE-ESTABLISHMENT OF INTESTINAL CONTINUITY*

ALBERT K. MINETA, RUDOLF O. F. OPPENHEIMER, HAROLD A. HARPER, ALLEN H. JOHNSON, FREDERICK M. BINKLEY, EDWIN E. KERR, COOPER DAVIS, AND H. J. MCCORKLE

These experiments were carried out to compare intestinal absorption in adult mongrel dogs that had undergone total gastrectomy followed by re-establishment of intestinal continuity by different methods. The experimental groups were arranged in accordance with the type of procedure used to reconstruct the gastro-intestinal tract, as follows: (1) esophagoduodenostomy, (2) esophagocoloduodenostomy, (3) esophagoduodenostomy with a doubled lumen jejunojejunostomy interposed between the esophagus and duodenum, (4) esophagoduodenostomy with a single jejunal loop interposed between the esophagus and duodenum and (5) esophagojejunostomy. Normal dogs and dogs with subtotal gastrectomy and gastrojejunostomy were used as controls.

METHODS

Carbohydrate absorption was studied by means of glucose tolerance tests performed in the following manner: One and one-half grams of glucose per pound of body weight were given by stomach tube in a concentration of 50 per cent glucose in water Samples of venous blood were taken when the animal was fasting and at intervals of 15 and 30 minutes and 1, 1½, 2, 3 and 4 hours after administration of glucose. A sodium fluoride–oxalate mixture was used as anticoagulant. Analyses for blood sugar were determined by the method of Somogyi.[3]

Absorption of incompletely hydrolyzed protein was studied following a test meal of 100 grams of a protein digest (Essenamine†) by hourly determinations of the plasma amino nitrogen concentration using the manometric ninhydrin–carbon dioxide method of Hamilton and Van Slyke.[1]

The absorption of fat was investigated by feeding, through a gastric tube, a test meal of 110 grams of butter, 100 cc. of condensed milk, 100 cc. of water and carmine powder, followed by the taking of hourly blood speci-

* From the Surgical Research Laboratories of the University of California School of Medicine, San Francisco. This study was supported by Cancer Research funds of the University of California

† An enzymatic digest of lactalbumin, supplied by Winthrop-Stearns, Inc.

obvious improvement in appearance of this segment from first to second operation left little doubt about rec
and congestion in the mesentery of
and division of the veins, but there
the normal and abnormal mesentery as between strangulated and normal bowel. The strangulated segment was the same thickness as the adjacent bowel in two experiments, and thicker than normal in the remaining two. The thickening was almost entirely due to hemorrhage, edema, and congestion in the muscularis. Subserosal hemorrhages were present in three experiments. The strangulated bowel was distended in two and smaller than the remaining obstructed segment in two.

The appearance of the mucosa was even more striking. When viewed from the mucosal surface, it was not possible to tell where the strangulated segment began and where the normal bowel ended.

Microscopic Appearance. The striking microscopic appearance of the strangulated segment contrasts sharply with those animals that did not have antibiotics.[5] The strangulated segment was almost entirely normal The superiority of Achromycin over Aureomycin under these conditions is suggested by the more nearly normal appearance of the Achromycin series.

The mucosa was normal in every instance, with only some slight edema in two specimens. The submucosa showed edema in all, and some congestion in two. The muscularis showed some edema and congestion in all, and scattered hemorrhages in two The contrast with the microscopic appearance in experiments without antibiotics[5] was one of the outstanding features of the experimental work.

CONCLUSIONS

1. Survival of dogs with strangulation obstruction can be obtained by placing antibiotics into the strangulated segment.
2. Hematemesis was reduced in comparison with animals that did not have antibiotic protection.
3. Fluid requirements were markedly reduced in antibiotic protected animals, the need for blood being most significantly reduced.
4. The peritoneal fluid rarely contained any bacteria, and clostridia were never recovered from the peritoneal fluid.
5 The peritoneal fluid from these animals remained pink, coagulable, non-hemolyzed and odorless throughout the period between operations.
6. Grossly and microscopically the strangulated segment was essentially normal at reoperation, showing that viability of the bowel had been maintained even though strangulation was created.
7. The mucosal surface of the strangulated segment remained normal
8 The importance of bacterial toxicity in strangulation obstruction has been further emphasized by obtaining survival with adequate antibacterial therapy restricted to the postoperative period

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studied. Furthermore, considerable differences in the fasting levels of blood lipid were found. For this reason the elevations in the blood lipids after administration of the test meal of fat were corrected for the fasting level in order to compare the results found in the different groups of animals. The

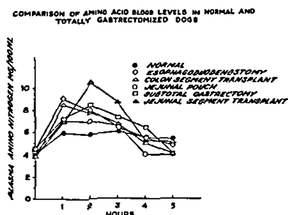


Fig 2. Graph showing a comparison of amino acid blood levels in normal and totally gastrectomized dogs

Fig 3 *A*, Deviation of total blood lipids from fasting level in normal dogs compared to dogs with subtotal gastrectomy and with esophagojejunostomy *B*, Deviation of total blood lipids from fasting level in normal dogs compared to those with esophagoduodenostomy and with colon pouches. *C*, Deviation of total blood lipids from fasting level in normal dogs compared to those with jejunal segment and with jejunal pouch.

mens for 10 consecutive hours to measure the lipid content of the blood. The hydroxamic acid method for analyses of long chain fatty acid esters was used to determine the blood lipids.[4]

RESULTS

After the administration of glucose there was a rise in the blood sugar of normal dogs which reached a peak in the 30-minute specimen, followed by a gradual progressive decline during the subsequent 3 hours. In the totally gastrectomized animals the peak of the blood sugar levels was very much higher than in the control animals, with a sharp drop during the succeeding 3 hours In some cases the blood sugar levels of the experimental animals fell below the fasting level in the second to third hour, although never to less than 40 mg. per 100 ml. Dogs with subtotal gastrectomy tended to show only a moderate rise in the blood sugar to levels which resembled those of the control animals, but which declined at a slightly faster rate after the first hour. The blood sugar levels of animals with a jejunal transplant exhibited the same rapid rise to a high peak as those animals with esophago-

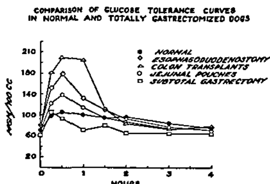


Fig 1. Graph showing a comparison of glucose tolerance curves in normal and totally gastrectomized dogs.

duodenostomies, though the peak was slightly lower. Animals in which a colon segment had been transplanted following total gastrectomy not only demonstrated the highest peak values, but the rate of decline in the blood sugar was also somewhat slower than that of any of the other animals. After [illegible]

occurrence of high levels of amino acids in the blood which were reached within 2 hours after the feeding of the test protein. This was followed by a return, at a steady rate, to normal levels. In contrast, the rise in the blood amino nitrogen levels of the normal dogs, while relatively slight, was maintained over the entire 5 hour period of observation These differences sug-
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Studies of fat absorption carried out by similar methods indicated that the rate of absorption was very slow and highly variable in all of the animals

LIVER AND PANCREAS

INTRODUCTION

WARREN H. COLE

In my estimation Thal and Brackney of Minnesota have made perhaps the most significant contribution to the etiology of acute hemorrhagic pancreatitis yet made. This was published elsewhere a few months ago. They showed that the Schwartzman reaction could be invoked in the pancreas by injecting a toxin of 44 B strain of meningococcus or *E. coli* into the pancreatic duct, and 24 hours later injecting the toxin intravenously in rabbits or goats. These animals were used because the Schwartzman reaction cannot be invoked in the dog. In practically all animals severe and fatal hemorrhagic pancreatitis was produced. Diffuse venous thrombosis is an important pathologic finding. At the Forum program Thal and Brackney have reported further on these experiments, to the effect that the underlying lesion of acute pancreatitis produced by the Schwartzman reaction is a fibrin-platelet capillary and vascular thrombosis associated with a local ischemia which in turn becomes an important factor in the necrosis. It is the most logical explanation of hemorrhagic necrosis which has ever come to my attention.

The role of secondary infection in the lethal outcome of acute experimental pancreatitis was emphasized in this Form program by Hara et al., of Arkansas, who found a decreased mortality rate in animals if antibiotics (Terramycin and neomycin) were given.

Keith and Watman, of Ohio, reported a significant or appreciable plasma volume deficit in the majority of patients with acute pancreatitis, and Elliott, also of Ohio, reported improvement in the mortality rate of animals with experimental acute pancreatitis when human serum albumin (treated by heating at 60° C. for 10 hours) was given.

Bonta, of Ohio, reports favorably on the use of retrograde pancreaticojejunostomy in the treatment of pancreatic duct obstruction produced experimentally in dogs. He reported equally good results with use of the Roux Y loop of jejunum or by direct suture of the pancreatic duct to jejunal mucosa The use of retrograde drainage of the pancreas in certain patients with obstructive lesions of the major pancreatic duct in the head of the pancreas is a distinct advancement in surgery of this organ, and may in certain cases stop the fibrosing destructive action resulting from obstruction of a major duct.

The use of cholografin for the production of cholangiograms is a very important contribution to surgery of the biliary tract, but I shall assume that it has been in use so long that all of you are familiar with it.

Scott and Vars, of Pennsylvania, have added to our knowledge of the role of the liver in fat metabolism by showing that in liver insufficiency (created by a biliary fistula or ligation of the common duct in rats) there is a decreased tolerance to fat administered intravenously.

Narat et al., of Illinois, reported a method of determining whether jaundice is obstructive or hepatogenous in character by injecting fluorescein

plasma lipid levels after feeding the fat meal showed only moderate increases, and these were attained slowly and persisted for several hours. The peak value for plasma lipid after oral feedings of a fatty meal was higher in the normal animals than in any of the operated animals with the exception of the subtotally gastrectomized group. In all of the experimental animals, the levels of fat in the blood at corresponding time intervals were usually lower than those of the controls, although the subtotally gastrectomized animals were again an exception In this group the plasma lipid levels were higher than in the controls, up to the last 3 hours of observation (Fig. 3).

SUMMARY

The absorption of glucose, amino acids and fat was investigated in dogs that had undergone total gastrectomy followed by reconstruction of intestinal continuity by different methods. Normal and subtotally gastrectomized animals served as controls. The studies were carried out by analyses of the blood sugar, plasma amino nitrogen, or total fatty acids at intervals after the feeding of a test meal of glucose, protein hydrolysate, or fat. In the gastrectomized animals the peak levels of glucose and of amino acid nitrogen in the blood were much higher than in the controls and the return to normal levels was more rapid. The plasma lipid levels after feeding the test meal of fat showed only moderate increases which were attained slowly and persisted for several hours. In all of the experimental animals with the exception of those with subtotal gastrectomy, the levels of fat in the blood at corresponding intervals were generally lower than in the normal animals.

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THE TURNOVER RATES OF PLASMA PROTEINS IN STATES OF LIVER INJURY, HEMORRHAGE, AND DIETARY DEPLETION*

ROBERT E. MADDEN**

The balance studies of Weech, Goettsch, and Reeves[1] and of Sachar, Horvitz, and Elman[2] have given valuable information concerning the relationship between plasma proteins and total body proteins in depletion. In the present work a dynamic approach to this relationship is attempted. The turnover rates of the plasma protein fractions albumin and total globulin are studied after labeling them with radioactive sulfur, S^{35}. Conditions of dietary depletion, hemorrhage, and liver injury are chosen as abnormal states of protein metabolism and their effects on the turnover, i.e., synthesis and utilization, of the proteins followed.

EXPERIMENTAL

Plasma Fractionation. Samples of fresh, heparinized plasma were separated into two fractions, albumin and total globulin, according to the method of Pillemer and Hutchinson.[3] Electrophoretic analyses of the fractions disclosed the albumin fraction to be over 80 per cent pure while the globulin fraction was virtually free of the faster moving component.

S^{35} Assay Procedure. Equal volumes of 20 per cent trichloroacetic acid (TCA) were added to the albumin solutions and the precipitate separated by centrifugation. The precipitates were then washed twice with 10 cc. portions of 10 per cent TCA. The globulin precipitates were treated twice with 10 cc. portions of 10 per cent TCA. The total sulfur of the plasma proteins and of urine was obtained as barium sulfate by a modification of the method of Benedict.[4] Protein precipitates were digested till clear in hot concentrated nitric acid prior to treatment with Benedict's reagent. The $BaSO_4$ precipitates were collected on tared paper disks using a Tracerlab filter funnel, dried overnight in a desiccator, and weighed. All samples were counted in an end window Geiger counter and corrected for self-absorption and radioactive decay.

Types of Experiments. Normal dogs were maintained on stock diets. On day zero they received a dose of S^{35} labeled yeast† mixed with dog meat. This yeast contains the majority of S^{35} activity as L-methionine and L-cystine. Blood samples of approximately 20 cc each were drawn periodically on succeeding days for about three weeks. The fall in specific activity of the protein sulfur, measured as $BaSO_4$, is a measure of the turnover of the protein. Hemorrhaged dogs were done the same as the normals except that on the sixth day they were subjected to a sudden massive hemorrhage of 50 per cent of the estimated blood volume. [illegible] to the same type of experiment underwen[illegible] foreign body (sponge) left beneath the liv[illegible] following sacrifice showed severe cholangitis and beginning cirrhosis. The transfused

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** The author wishes to acknowledge the valuable technical assistance of Mrs Ruth McGrath and Mr. E Hoppe.

† Obtained from the Division of Radioactive Pharmaceuticals, Abbott Laboratories.

into the common duct. Since this dye is absorbed from the intestinal mucosa but not from the common duct or gallbladder, it can be detected in the blood stream 3 minutes after injection into the common duct, if the duct is open, but will be absent if the common duct is obstructed.

Ellison and associates, of Ohio, have offered another test to differentiate hepatogenous from obstructive jaundice. They determined the mucoprotein level in the blood and found that in patients with hepatitis the average level was 27 mg. per cent, whereas in patients with extrahepatic obstructive jaundice the average level was 107 mg. per cent. It would appear to me that this test offers fine promise of improving our diagnostic ability in jaundice, particularly from the differentiation standpoint.

I would like to comment on the future and feasibility of hepatectomy. We now know removal of a lobe of the liver is possible, and with a reasonably low mortality rate Wangensteen, Pack and others have reported successful cases. By ligating the artery and vein to the involved lobe before its excision, the vascularity is reduced to a minor factor. I think the operation has a definite place in the treatment of hepatomas and slow-growing tumors of that type, but I am not very hopeful that removal of a liver lobe for a metastatic lesion will be curative. Yet, I am thoroughly convinced that it should and must be tried in certain cases until we get an answer to this question

a first order type of process, is obtained with the data thus treated. It will be noted that there is a considerable range of albumin half life between the two normal dogs. This variation between individuals is to be noted in the data of Sterling,[5] who determined albumin turnover rates in a series of normal human subjects. Figure 2 illustrates the decay curve of a dog subjected to hemorrhage. There was no effect on either the position or the slope of curves of albumin and globulin in any of the dogs subjected to hemorrhage. Further, the turnover rates for both fractions of the plasma proteins were all within normal range.

All of the transfused dogs tended to show a slightly accelerated turnover rate. The protein depleted dogs showed the same rates as the normal. The apparent increase in turnover rate of dogs receiving transfusions of radioactive blood is undoubtedly due to less reutilization of isotope. Isotope released by the breakdown of radioactive protein enters the circulating pool

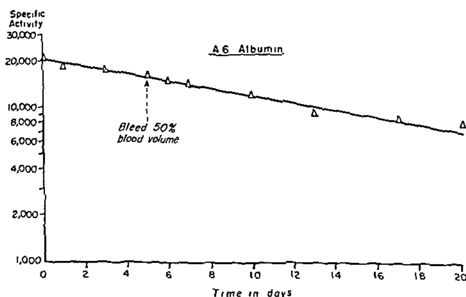


FIG. 2 Turnover curve of a dog subjected to hemorrhage. No alteration of the curve is effected by the hemorrhage.

of protein precursors and is subject to reutilization, giving falsely high values of specific activity and apparently longer half lives. When a dog is fed isotope it goes to all body proteins and a much greater amount is available for reutilization than if only isotope labeled blood proteins are injected. The figures for dog A-13 must therefore be accepted as being closer to the true turnover rate.

Figure 3 illustrates the levels of excreted urinary radioactivity following feeding of isotope to a normal dog. This curve is *non-logarithmic*, in contrast to the others. This indicates contribution of sulfur to urinary sulfate from several different sources, each with a [illegible]

Some idea of the size [illegible] obtained by determining [illegible] injection of radioactive [illegible] time, then determining the biologic decay curve starting from day one in the usual manner. When the latter is extrapolated back to zero time it is found that the actual zero time value is

dogs on day zero received a transfusion of 250 cc of S^{35} labeled whole blood from donor dogs previously fed S^{35} labeled yeast. During transfusion an equal volume of blood was drawn from a contralateral vein. Two of the transfused dogs were protein depleted for nine (A-3) and six (A-14) weeks prior to transfusion by maintaining them on a diet suggested by Weech, Goettsch, and Reeves.[1] This provides a maximum of 1.23 gm. nitrogen per day. All dogs were of approximately equal weight. On some dogs the total urine output was collected.

RESULTS AND INTERPRETATIONS

The turnover rates for the various types of experiments are listed in Table 1. These figures are obtained from graphs such as Figure 1, with the logarithm of specific activity plotted against time. A straight line, indicating

Table 1. Turnover Rates for Various Experiments

TYPE DOG	EXPERIMENT NUMBER	HALF LIFE IN DAYS		PERCENTAGE DAILY TURNOVER	
		ALBUMIN	GLOBULIN	ALBUMIN	GLOBULIN
Normal	A-1	13 0	8 3	5 3	8 3
	A-10	19 5	7 9	3 5	8 7
Cirrhotic	A-2	15 6	8 4	4.4	8 2
Hemorrhaged	A-6	13.0	8.7	5 3	8.1
	A-11	13.1	8 2	5 3	8 4
	A-12	12.6	8 0	5 5	8 6
Normal Transfused	A-13	10 2	6 6	6.8	10 5
Depleted Transfused	A-3	9 0	5 6	7 7	12 3
	A-14	11 5	6 9	6 0	10 0

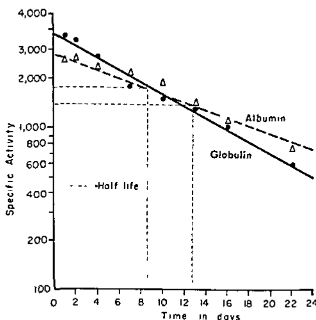


Fig. 1. A typical turnover curve, dog A-1, for albumin and globulin. Specific activity is plotted logarithmically against time Figure illustrates method of graphically determining half life.

DISCUSSION

The protein turnover rate represents the rate of utilization of that protein. It is obtained from the graphically determined half life by the expression

$$\text{Turnover rate} = \frac{\ln 2}{t_{1/2}} \times 100.$$

Using the data from dog A-13 as normal this means that 6.8 per cent of total albumin and 10.5 per cent of total globulin is utilized daily by the dog. The data in Table 1 indicate that severe protein depletion, at least over a few weeks' time, does not alter this rate of utilization significantly. However, it should be pointed out that these calculations are based on the assumption of a steady state. Actually the total protein pool of a depleted dog is diminishing slightly over the period of the experiment. The replacement of labeled with non-labeled protein could be occurring at a slightly diminished rate. Moderate liver damage is also apparently without effect.

The sudden loss of approximately one-half of the circulating proteins by hemorrhage also failed to disclose alterations in albumin or globulin turnover rates. Madden and Gould[6] had previously described the same result with fibrinogen in the dog. This means that whatever proteins or protein precursors are mobilized to replace this loss, they are turning over at the same rate as the circulating proteins, and that under the conditions employed there was no acceleration of protein synthesis. Much of this mobilized protein must be present as lymph protein Co Tui et al.[7] have described an increase in thoracic duct lymph protein following hemorrhage in the dog Madden and Whipple,[8] using the technique of repeated plasmapheresis, have demonstrated that such reserve pools exist and are probably quite large. They suggest that a portion of the intercellular body proteins are available for plasma protein replacement. The present data are consistent with this view. Hemorrhagic and dietary depletion either fail to alter protein synthesis rates or the reserve pools provide so large a buffer that the experimental conditions failed to elicit a response.

Yuile et al.[9] have presented evidence showing the conversion of parenterally administered plasma proteins to tissue protein without evidence of breakdown. If the prospective surgical patient in a state of protein depletion is to be repleted rapidly it would appear that the injection of large amounts of plasma proteins would be an adequate vehicle for achieving this. First, the plasma proteins are in rapid equilibrium with a larger pool of proteins, lymph and tissue, and provide a funnel through which body proteins could be enriched Secondly, the slow rates of albumin and globulin turnover in the depleted or the normal probably preclude a rapid replacement via the dietary route. The effects of parenterally administered protein hydrolysates on turnover rates deserve further study.

SUMMARY

1. Approximate normal daily turnover rates of 6.8 per cent for albumin and 10 5 per cent for globulin in the dog have been determined following the injection of S^{35} biosynthetically labeled whole blood. Smaller values are obtained if the isotope is fed; this is attributed to reutilization of isotope.

2. Dietary protein depletion, moderate liver injury, or sudden massive hemorrhage do not detectably affect these turnover rates.

higher than that found by extrapolation. This difference is a measure of the mixing of injected intravascular labeled proteins with extravascular unlabeled proteins. The percentage of total albumin or globulin present within the vascular tree can be calculated by simple isotope dilution:

$$\text{Percentage intravascular} = \frac{\text{Extrapolated zero time S.A.}}{\text{Determined zero time S.A}} \times 100.$$

Table 2 gives the results as determined on three dogs. It is seen that in the normal roughly half the total albumin and globulin are found intravascularly at any one time, whereas in depleted dogs apparently the extravascular pool is diminished.

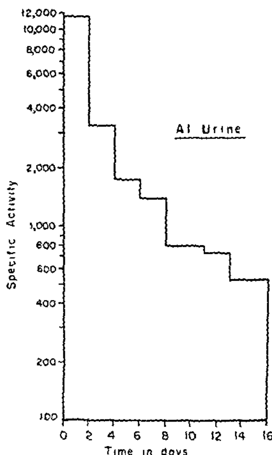


Fig 3. Urinary levels of radioactivity in a normal dog fed S^{35} labeled sulfate.

Table 2. Percentage of Total Albumin and Globulin in Vascular Tree

TYPE DOG	EXPERIMENT NUMBER	PERCENTAGE OF EXCHANGEABLE POOL IN VASCULAR TREE: ALBUMIN	GLOBULIN
Normal transfused	A-13	66	46
Depleted transfused	A-3	87	83
	A-14		77

gm. in weight, were used. These animals were fed a standard synthetic diet containing 18 per cent casein and 3 per cent fat.[3] Animals which were prepared by bile duct cannulation were given 1 per cent saline to drink following operation.

Bile fistula animals were prepared by the method of Fisher and Vars.[4] These animals were allowed to stabilize for four days following operation before receiving fat emulsions. Twenty-four hour samples of bile were collected in latex bags. These samples were analyzed for cholic acid by the method of Irvin, Johnston, and Kopala[5] and for cholesterol by the method of Foldes and Wilson.[6] Bile duct occlusion was accomplished by double ligation and transection of the common bile duct. The subcutaneous injection of chloroform, 1.2 ml. per kilogram of body weight, dissolved in mineral oil, was used to produce sublethal chloroform intoxication. Animals prepared for portal vein infusions were cannulated with small polyethylene tubing. These cannulae were inserted through a small mesenteric tributary of the portal vein and passed distally until the tip of the cannula was in the intrahepatic portion of the vein. The cannula was brought out through the abdominal wall and subcutaneously to the dorsal midline of the animal. Infusions were carried out with a constant infusion apparatus at a constant rate of 0 75 ml. per hour.

Three preparations of fat emulsion were used, 10 per cent sesame oil,* 15 per cent olive oil,† and 50 per cent coconut oil.‡ The amount injected varied from 1 0 to 5.0 mg of fat per gram of body weight. The disappearance rates of the infused fat emulsion were determined by the turbidimetric method of Geyer, Mann and Stare.[7]

In determining the rates of disappearance, 2 5 mg of fat per gram of body weight was injected. The emulsion was injected into the femoral vein under light ether anesthesia Tail vein samples were then taken at 5, 20, 60, and 120 minute intervals, for turbidimetric analysis

The animals were sacrificed at the end of each experiment and tissue specimens were taken from the liver, spleen, and lung. Frozen sections were prepared from the Formalin-fixed tissues and stained for fat.

RESULTS

When emulsified fat was injected intraperitoneally into normal animals, the animals gained weight and maintained a normal food intake. At the end of the 7 and 14 day periods, examination revealed no residual fat and no peritoneal irritation. Fat emulsion administered in the same manner to bile fistula animals produced a striking contrast. These animals showed progressive weight loss, diminished food intake, and gradual deterioration. Examination revealed a marked peritoneal reaction to the injections and a variable amount of unabsorbed thick, fatty material.

As can be seen in Table 1, when emulsified fat was administered by tail vein to the bile fistula animal, it was tolerated much better. The weight loss was not great and the food intake was not diminished. There was no evidence of toxicity. The amount of calories provided by the injections was not sufficient to show any effect over the short experimental period.

Fat emulsion infused into the portal vein of three normal animals was

* Merck & Co , Inc., Rahway, N. J.
† Lipomul, The Upjohn Company, Kalamazoo, Michigan
‡ Schenley Laboratories, Inc , Lawrenceburg, Indiana.

3. A rough estimation of the percentage of the exchangeable protein pools present intravascularly has been made. This percentage tends to be higher in the depleted dog.
4. The implications of these data with respect to repletion of the depleted patient have been discussed.

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RESPONSE OF ANIMALS WITH BILIARY FISTULA, BILE DUCT OCCLUSION, OR CHLOROFORM INTOXICATION TO PARENTERAL FAT FEEDING*

STEWART M. SCOTT AND HARRY M. VARS

Variations in diet, especially that of protein, influence the excretion of bile in the chronic bile fistula animal. Most experiments by necessity have been carried out on animals fed a diet low in fat content. High concentrations of dietary fat in the chronic bile fistula rat cause diminished food intake, increased weight loss, fatty stools, and gradual deterioration of the animal The mechanism whereby bile aids in the absorption and utilization of fat from the intestines is not completely understood.

The administration of fat emulsion intravenously and intraperitoneally to the normal rat has been reported.[1, 2] These studies indicated that at least the normal rat could utilize fat given by these routes We were interested in knowing whether or not the animal with an abnormal enterohepatic bile circulation could utilize parenterally administered fat emulsions equally well. If this was true, we hoped to learn something of the effect of fat on the production of bile in the chronic fistula rat.

Our experiments have produced doubt that parenterally administered fat emulsion in these animals results in a normal response There is no significant effect of intravenous fat emulsion on the excretion of cholesterol or cholic acid in the bile fistula rat There may be some intolerance to the fat emulsion, similar to that found in animals given high fat diets.

METHOD

In each experiment adult male Wistar rats, averaging approximately 250

* From the Harrison Department of Surgical Research, Schools of Medicine, University of Pennsylvania, Philadelphia This work was supported in part under contract between the Department of the Army and the University of Pennsylvania, and in part by the Merck Institute for Therapeutic Research

emulsion in the four groups of animals. The most striking difference occurred in the animals previously treated with chloroform. Within one hour 95 per cent of the emulsion had disappeared from the circulation. There was a slight delay in the rate of disappearance in the bile duct occluded animals. There was no difference in the rate of disappearance in the bile fistula animals as compared to the normal animals.

Table 3 shows the effect of intravenous fat emulsion on biliary excretion

Table 3. The Effect of Intravenous Fat Emulsion on Average Daily Biliary Excretion

ANIMALS NO.	WEIGHT GM.	BILE VOLUME ML	CHOLIC ACID* MG./24 HR	CHOLESTEROL* MG /24 HR	RATIO CA/C	AMT. OF FAT MG /GM	DAYS NO.
4	240	13 6 (±2 7)	41.5 (±9.0)	1 98 (±0 33)	21.2	0	6
5	249	14 7 (±1.8)	42 2 (±6 0)	1 66 (±0 31)	26 8	2 5	6
4	242	17 0 (±2 4)	39 4 (±4 5)	1.80 (±0 41)	25 1	5 0	4

* The values are given as mg./24 hr plus or minus the S.D from the mean. CA = cholic acid C = cholesterol

in the chronic bile fistula animal. There was no change in the excretion of cholic acid and cholesterol. The bile volume was not significantly increased.

DISCUSSION

The failure of the bile fistula rats to tolerate intraperitoneal fat emulsion suggests either that a normal flow of bile into the intestines is necessary for the proper utilization of the fat, or that *these animals, subjected to the stress* of operation, were less able to tolerate the administration of the fat emulsion. These observations are supported by the inability of the rats with bile ducts [illegible] the bile [illegible] ein. This [illegible] is really significant Bile is active in the emulsification of fat within the intestine and aids pancreatic lipolysis.[8] Whether or not there is a *more intrinsic* function of bile can certainly not be concluded from these experiments. It does appear that the bile fistula animals were less able to withstand any reaction that may have been produced with the administration of fat emulsions *by parenteral routes.*

To observe the effects of fat emulsion on biliary excretion, perfusion of the liver directly by way of the portal vein was attempted. The bile fistula animals in this group had the greatest incidence of reaction and death The explanation of these deaths can not be attributed to the usual causes of reaction to fat emulsions.

Removal of various organs, especially the liver, has been shown to prolong the rate of disappearance of intravenous fat from *the circulation*[9] When the liver is severely damaged by chloroform, there is a marked increase in the rate of disappearance of intravenous fat emulsion. The rate of disappearance is greatly influenced also by the clearing factors present in the blood.[10] Whether or not the plasma from these chloroform intoxicated animals has

tolerated quite well. A fourth normal animal died following a single infusion. All of the bile fistula animals died following the portal vein infusions of fat emulsion Examination of these animals revealed pale swollen livers with a moderate amount of extracellular fat particles. The spleens were similarly enlarged and contained a large amount of fat particles

The bile duct ligated animals were also uniform in their failure to absorb and utilize intraperitoneal fat emulsion. Weight loss and death occurred rapidly. At death the peritoneal cavity was filled with a thin milky material

Table 1. Comparison of the Effect of Fat Emulsions on Normal Rats and Rats with Chronic Bile Fistulae, Ductal Occlusion and Sublethal Chloroform Intoxication

ANIMALS		TYPE OF FAT	INJECTIONS			REACTION TO
TYPE	NO	%	MG /GM	NO	ROUTE*	INJECTIONS†
Normal	3	10	1 0	14	IP	0
	3	50	2 5	7	IP	0
	6	15	2 5	6	IV	0
	4	50	5 0	4–5	IV	0
Bile fistula	7	10	2 0	7	IP	++
	9	50	2 5	7	IP	++
	8	15	2 5	4–8	IV	++
	5	15	5 0	6–11	IV	+
	7	50	5 0	1–3	PV	++
	4	15	2 5	4	PV	+
Ductal ligated	10	50	2 5	5	IP	++
Chloroform intoxicated	6	15	2 5	1	IV	+

* IP, intraperitoneal injection, IV, injection by tail or saphenous vein, PV, infusion of the portal vein

† 0, no reaction, +, minimal reaction, ++, severe reaction

In two animals approximately 50 per cent of the injected fat was recovered from the peritoneal cavity.

The chloroform treated animals were given a single intravenous injection of fat emulsion. Two of the six animals survived longer than 24 hours following the injection. Microscopic tissue examination from these animals revealed a marked extracellular infiltration of fat within the liver and spleen

Table 2 compares the rates of disappearance of intravenously injected fat

Table 2. A Comparison of the Clearance Rates Following the Intravenous Injection of Fat Emulsion

GROUP	ANIMALS NO	% REMOVED IN MINUTES* 20	60	120
Normal	7	49 8 (±11 1)	66 2 (±16 8)	69 5 (± 9 8)
Bile fistula	7	56 0 (±14 9)	61 7 (± 6 2)	
Ductal occluded	5	15 7 (±10 5)	52 2 (±12 3)	73 0 (±13 5)
Chloroform intoxicated	4	85 0 (± 2 5)	95 0 (± 4 1)	97 0 (± 2 2)

* The values are given as % plus or minus the standard deviation from the mean

FLUORESCEIN INJECTION INTO EXTRAHEPATIC BILIARY DUCTS FOR DIFFERENTIAL DIAGNOSIS OF OBSTRUCTIVE AND NON-OBSTRUCTIVE JAUNDICE*

JOSEPH K. NARAT, JOSEPH P. CANGELOSI, AND JOHN V. BELMONTE

Approximately one-third of all icteric patients present baffling diagnostic problems.[1] If the history and clinical examination, aided by the conventional biochemical tests, are unable to establish the precise diagnosis in a jaundiced patient, exploratory celiotomy is indicated.

Unfortunately even direct inspection and palpation of the involved organs may fail to disclose the true nature of icterus because intervening parenchymal changes following extrahepatic obstructive jaundice of long standing may simulate a primary hepatocellular lesion and, conversely, in cholangiolitic hepatitis an element of obstruction can be created by stasis in the bile capillaries. In such instances the surgeon is confronted with the problem whether to open or not to open the common duct. While exploration of the common duct is imperative in obstructive jaundice, the procedure is not only superfluous but hazardous in hepatogenous jaundice. As Glenn[2] pointed out, in spite of modern chemotherapy, employment of vitamin K, improved methods of anesthesia and better understanding of water, electrolyte and nutritional requirements, choledochotomy carries an additional risk and should be done only in case of a specific indication.

It follows that a test which would help to distinguish obstructive from non-obstructive jaundice in course of an exploration would be desirable.

With this thought in mind the authors have developed the following test based on the assumption that fluorescein dye is rapidly absorbed by the intestinal mucosa but only slowly if at all by the extrahepatic biliary system. The accumulated experimental evidence lends support to this hypothesis.

In the first series of experiments 2 cc of a 5 per cent commercial fluorescein dye† was injected in 10 dogs after aspiration of bile into either the gallbladder or the cystic or the common duct, and blood samples were obtained from an exposed femoral vein 2, 3, 6, 8 and 10 minutes after the injection To prevent absorption by the peritoneum of inadvertently spilled dye, the needle with the syringe attached was kept in situ until blood samples were obtained and, as a further precaution, sterile cotton was placed around the needle. The blood specimens were centrifuged and the test tubes were inspected under ultraviolet light. The supernatant serum showed brilliant fluorescence in all specimens, easily recognizable, although in a lesser degree, also when the tube was viewed in bright sunshine against a dark background

In the second series of 10 dogs obstruction was created by one of the following methods: complete severing of the common duct between 2 ligatures, clamping the duct with a hemostat or insertion of a plug of mold-

* [illegible]
† [illegible]
N. Y [illegible]

an increased clearing factor is not known. The only conclusions which can be made from a comparison of the disappearance rates in our animals are that diversion or occlusion of the bile flow into the intestines does not alter the rate of disappearance significantly, whereas injury to the liver by chloroform does affect the rate of disappearance.

Intravenous fat emulsion had no effect on the biliary excretion of cholesterol or cholic acid These findings are in agreement with those of Grossman[11] and of Virtue[12] Virtue found that in bile fistula dogs maintained only on intravenous olive oil emulsion for three days, there was a gradual decrease in the excretion of cholic acid.

SUMMARY AND CONCLUSIONS

1. Bile fistula, bile duct occluded, and chloroform intoxicated rats, had *a greater incidence of reaction to parenteral fat emulsion than normal* rats.
2. The rate of disappearance of intravenous fat emulsion from the blood stream was not altered in the bile fistula or bile duct ligated rats.
3. Chloroform caused an increase in the rate of disappearance of emulsified fat from the circulation, as measured by a turbidimetric method.
4. Intravenous fat emulsion given over four and six day periods did not significantly alter the biliary excretion of cholesterol or cholic acid.

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SERUM MUCOPROTEIN LEVEL AS AN AID TO DIFFERENTIAL DIAGNOSIS IN JAUNDICED PATIENTS*

EDWIN H. ELLISON, ROGER D. WILLIAMS, RICHARD O. MOORE, AND ROBERT M. ZOLLINGER

The differential diagnosis in jaundiced patients continues to present a challenge to both surgeon and internist. As a matter of fact, during the period from July, 1949, through July, 1953, hepatocellular disease was found in 11 out of 100 consecutive patients operated at University Hospital with the diagnosis of obstructive or surgical jaundice.[1] Inasmuch as one-half of these 11 patients had laboratory evidence strongly favoring obstructive jaundice and five of them had gallstones by x-ray, the incidence of incorrect diagnosis might seem justified. On the other hand, surgery had nothing to offer these patients except an increase in morbidity and possible mortality. The diagnoses established by formal liver biopsy included hepatitis, biliary cirrhosis and cholangitis.

In view of these findings we were considerably interested in the suggestion of Greenspan and Dreiling[2] that the determination of serum mucoproteins might aid in the differentiation of hepatocellular from obstructive jaundice. Mucoproteins are a carbohydrate-rich alpha globulin comprising approximately 1 per cent of the total serum proteins. They have been studied in a wide variety of clinical entities and rather consistent elevations have been reported in patients with malignancy[3] and infectious processes.[4] Reduction in the serum levels has been found in diabetes and certain endocrine disturbances[5] and, most important, in hepatocellular disease.[6]

During the period, October, 1953, through September, 1954, serum mucoprotein levels were determined in 400 patients on the surgical service of the University Hospital. The serum mucoprotein level was estimated after the method of Winzler,[7] by differential precipitation of serum proteins from mucoproteins with perchloric and phosphotungstic acids and the mucoprotein reported as the casein equivalent, utilizing the biuret peptide reaction.

All told, 139 normal subjects (i.e., non-hospital controls and patients admitted for elective surgery with no evidence of infectious, neoplastic or endocrine disease) have been studied. The average mucoprotein level was 53.3 mg. per cent and no significant variation was noted between males and females. The mean for the 65 males approximated 54 mg. per cent (S.D. ±10.77), and for the 74 females, 52.7 mg. per cent (S.D. ±12.62) (Table 1). Those values below 40 mg. per cent occurred in technicians and medical student controls under the age of 25 years; no other age variation according to decades was noted.

As reported by others, the serum mucoprotein concentration was increased above normal in about 75 per cent of the patients with malignancies and in nearly all patients with infections. No specificity was noted.

In 100 patients with jaundice, the serum mucoprotein concentration

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ing clay into the duct. Fluorescence was absent in the serum of all blood specimen obtained 2, 3, 6, 8, 10 and 15 minutes after injection of fluorescein into the duct.

In the third series of 10 dogs a few drops of the dye were intentionally spilled into the peritoneal cavity. Rapid absorption of the dye was demonstrated by the presence of fluorescence in blood specimens obtained as early as 2 minutes after instillation of the dye into the peritoneal cavity

In 2 patients with intact cystic and common ducts and in 2 other patients with extrahepatic obstruction injections of dye confirmed experimental findings in dogs.

COMMENTS

The tests show that fluorescein dye is readily absorbed by the intestinal mucosa and by the parietal peritoneum but not by the extrahepatic biliary system Hence a positive test, meaning fluorescence of the serum after injection of the dye into extrahepatic ducts, is of no diagnostic value, because it may occur in the presence of (1) intact ducts, (2) partial obstruction and even (3) complete obstruction if some of the dye is spilled and absorbed by the peritoneum On the other hand, a negative result, or absence of fluorescence of the serum serves as incontrovertible evidence of obstruction Although the nature of obstruction—a stone, postoperative stricture, tumor, abscess, collection of bile around the ducts or sclerosing pancreatitis —is not revealed in the test, the negative result is indicative of occlusion of the ducts and therefore suggests the necessity of further exploration.

SUMMARY

Absence of fluorescence in the serum of a blood sample obtained 3 minutes after injection of fluorescein dye into the gallbladder or the cystic or common duct is a definite proof of extrahepatic obstruction.

Positive test, or fluorescence of the serum, has no diagnostic value because it may occur in the presence of intact ducts as well as a partial obstruction.

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ranged from 68 to 183 mg. per cent, with a mean of 109 89 mg. per cent (S.D. ±31.3). Six of the nine cases had values greater than 100 mg. per cent, while only five of the 55 extrahepatic obstructions exceeded this value. Further studies will be required to determine if this finding is to be of clinical value.

In those patients with hepatitis* the mucoprotein concentration ranged from 16 to 62 mg. per cent with a mean value of 35.5 mg. per cent (S.D. ±11.72) and represented a marked reduction from normal. Serum mucoprotein was diagnostically low in one out of two patients with a one plus cephalin, six out of ten with a normal A/G ratio, and four out of five with a thymol less than 30. Seven patients had abnormally elevated alkaline

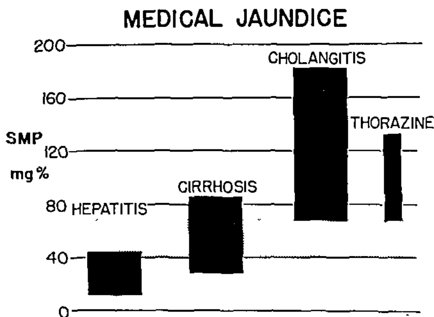


FIG 2 In 19 patients with hepatitis, the mucoprotein concentration ranged from 16 to 62 mg per cent with a mean value of 35 5 mg. per cent (S D ±11.72), and represented a marked reduction from normal. The highest values were observed in 9 patients with intrahepatic obstructive jaundice resulting from cholangitis.

phosphatases and among these the SM concentration was less than 40 mg per cent in four instances.

The serum mucoprotein determinations would seem, therefore, to be useful in differentiating obstructive from hepatocellular jaundice. It must be remembered, however, that superimposed or complicating infections, malignant disease and endocrine dysfunction may alter the laboratory findings and must be considered in interpreting the serum concentrations. For example, five of the 19 hepatitis patients had serum mucoprotein concentrations above 40 mg. per cent (Fig 3). Of these, two patients had carcinoma of the cervix. An additional two patients were found to be recovering from some other infectious process. All told, there was only one instance wherein the findings could not be explained satisfactorily.

* In the absence of any specific biologic test for hepatitis, the diagnosis was of necessity based on clinical findings, laboratory evaluation of liver function and the subsidence of jaundice under medical management.

varied markedly depending upon the pathogenesis of the disease. The SM levels were elevated above normal in the great majority of 55 patients with extrahepatic obstructive jaundice, regardless of etiology (Fig. 1). The mean value was calculated to be 80.4 mg per cent (S.D. ±31.86). Serum mucoprotein concentrations less than 40 mg. per cent were noted in five instances, and of these, three had an associated diagnosis of hyperthyroidism and one was a known diabetic. The laboratory data in the remaining exception, a patient with terminal carcinoma of the pancreas, indicated an

Table 1 Serum Mucoprotein Concentrations in Normal Subjects and in Patients with Infections or Malignancy

GROUP	SEX	TOTAL PATIENTS STUDIED	SERUM MUCOPROTEIN CONCENTRATIONS (MG %)* RANGE	MEAN (S D)
Normal subjects	Male	65	32– 79	54 0 (±10 77)
	Female	74	29– 78	52.7 (±12 62)
Patients with infection	Male	46	41–199	94 1 (±34 51)
	Female	76	30–176	86 4 (±31 89)
Patients with malignancy	Male	13	34–228	94 6 (±44 77)
	Female	26	50–169	92 3 (±29 39)

* Reported as casein equivalents

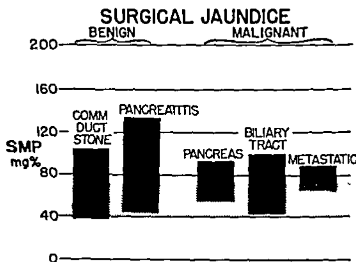


Fig. 1 The serum mucoprotein concentrations were elevated above normal in the great majority of 55 patients with extrahepatic obstructive jaundice, regardless of etiology.

advanced degree of hepatic damage which may account for the exceptional low value of 24 mg per cent Although the average rise from malignancy exceeded that for common duct stone, the highest values occurred in benign stricture and for the most part varied directly with the duration and degree of obstruction.

SERUM MUCOPROTEIN IN MEDICAL JAUNDICE

The highest SM values were observed in nine patients with intrahepatic obstructive jaundice resulting from cholangitis (Fig. 2). The concentration

operated were found to have non-surgical jaundice. This represents a considerable improvement in diagnostic acuity. In one of these the alkaline phosphatase was markedly elevated without other laboratory evidence of parenchymatous disease. Hepatitis plus cholangitis was found. The second patient was of interest in that she had first noted jaundice while receiving Thorazine. With this history exploration was postponed for four weeks even though all laboratory data supported obstructive jaundice. On the day of operation the icteric index approximated 150, thymol was negative and cephalin was reported as one plus. The alkaline phosphatase had reached a level of 47.5. The mucoprotein was a high normal. The gallbladder was nearly empty of bile and the common duct was of normal size. The entire biliary tract was visualized by operative cholangiography and no obstruction was demonstrated. Microscopic sections of the liver showed blockage of the small intrahepatic biliary channels with what appeared to be precipitated bile pigment. There was no evidence of inflammation.

CONCLUSIONS

The uniformly low findings in hepatocellular disease as compared to the consistent elevation seen in extrahepatic obstructions (Fig. 4) and the marked elevation in intrahepatic obstructions (cholangitis) indicate that a knowledge of the serum mucoprotein concentration may be of value in differentiating medical from surgical jaundice when considered with other clinical and laboratory findings.

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The SM values in biliary cirrhosis showed rather wide variations and were not as low as has been reported in the current literature. For the most part, however, these patients presented themselves with severe complications of their disease (i.e., massive hemorrhage, etc.), which probably explains the wide range of values. It seemed wise, therefore, not to attempt further analysis of this group.

INFLUENCE ON OPERATIONS FOR JAUNDICE

During the period of this study only two patients out of 57 (3 5 per cent)

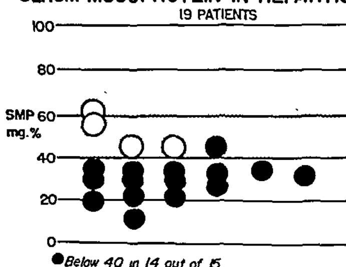


Fig 3 Five of the 19 hepatitis patients had SM values above 40 mg per cent Of these, two patients had carcinoma, and two patients were recovering from some other infectious process All told, there was only one instance where the high value could not be explained

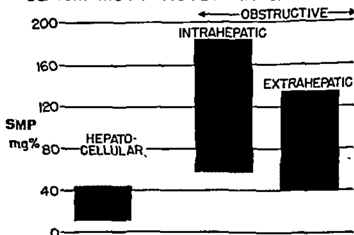


Fig 4. The marked variation in response to serum mucoprotein in obstructive jaundice as compared to hepatocellular jaundice offers an additional method for differentiating these disease processes.

erated water at 1° to 2°C. was circulated continuously. Body temperature was measured by the insertion of a mercury bulb thermometer high in the rectum. Shivering was controlled by the use of small supplemental doses of veterinary Nembutal. It required 2½ to 3 hours to reduce rectal temperatures to 25°C., and following this the temperature continued to fall 2° to 3°C. spontaneously. Frequent observations of pulse rate, respirations, and level of consciousness were made. At 32°C. the animals were connected to a mechanical respirator and ventilated with 100 per cent oxygen to prevent anoxemia. During this period of induced hypothermia, the continuous infusion of BSP was maintained, but it was necessary to decrease gradually the rate of infusion in order to avoid a progressive increase in blood Bromsulphalein levels. On the basis of preliminary experiments, it was decided to reduce the rate of infusion approximately 5 per cent for each 1°C reduction in body temperature. In this manner, it was possible to maintain relatively constant the peripheral concentration of Bromsulphalein. Appropriate samples of peripheral and hepatic venous blood were drawn simultaneously at 3° to 4°C. decreases in body temperature. Hematocrit values were obtained prior to cooling, and at the completion of the cooling process. An adjustment at 23°C. is made in the calculation for any changes observed in hematocrit due to hemoconcentration.

Liver function tests were carried out in all experiments. These included cephalin flocculation, total bilirubin, BSP clearance, T.P., A/G and prothrombin time. Baseline tests were carried out prior to cooling, and repeated three days and three weeks later. Animals exhibiting abnormalities of function in the baseline tests were discarded.

OBSERVATIONS

Splanchnic Blood Flow. A total of eight animals completed the experiments, although a number of others were discarded as incomplete for a variety of causes The experimental results are recorded in Table 1. This is a tabular summary of all completed experiments. Table 2 is a detailed account of a single experiment. Splanchnic blood flow decreases progressively in a linear fashion as the body temperature falls. Recorded graphically, the progressive decrease in splanchnic blood flow parallels the fall in cardiac output and oxygen consumption in hypothermic animals. Bigelow[3] has recorded the changes in both cardiac output and oxygen consumption in the hypothermic dog, and has found these to approximate 15 per cent of normal in the dog at 20°C.

Table 1. Splanchnic Blood Flow at Reduced Body Temperature (ml./min.)

DOG NO	TEMPERATURE, °C.				
	37	33	29	26	23
53–332	603	491	382	295	190
53–409	729	641	490	304	196
54–130	524	491	326	151	92
53–342	489	400	312	111	86
52–105	402	329	264	163	101
53–26	565	485	361	181	110
53–359	429	351	230	148	72
53–331	536	401	291	216	98

EFFECT OF DECREASED BODY TEMPERATURE ON LIVER FUNCTION AND SPLANCHNIC BLOOD FLOW IN DOGS*

E. BRUCE HALLETT

Current interest in hypothermia has resulted in an ever-increasing understanding of the alterations in basic physiologic processes which occur at reduced body temperature. This work was initiated in order to evaluate changes in liver function and to record alterations in splanchnic blood flow in dogs under hypothermia. The results appear to add further documentation of the ability of the organism, and the liver in particular, to adapt to this environmental change. Investigation of liver function failed to demonstrate abnormalities of function following hypothermia despite the marked reduction in splanchnic blood flow recorded. No important side effects or sequelae were observed in relation to hepatic function in the post-cooling period.

METHODS

Adult mongrel dogs of medium size were used in these experiments. The animals were prepared for cooling by close clipping and were anesthetized with veterinary Nembutal in accordance with body weight. As each animal was to serve as its own control, a series of baseline observations on splanchnic blood flow were made prior to cooling These were carried out in accordance with the Bromsulphalein clearance technique described by Bradley.[1] In the early groups of experiments, a ureteral catheter was inserted into a jugular vein and guided under fluoroscopic control into a hepatic vein in order to obtain samples of hepatic venous blood. In later experiments, the hepatic vein was cannulated under direct vision in accordance with the method described by Grindley[2] This was carried out aseptically several days in advance of the cooling procedure, and a small vinyl catheter leading from the hepatic vein to the exterior was maintained patent with daily instillations of small amounts of heparin The operative placement of the catheter proved to be a decided advantage over the fluoroscopically controlled group, as blood samples were more readily obtained This is probably related to the general vascular constriction which occurs under hypothermia, and which made the withdrawal of blood samples through a long ureteral catheter difficult, and at times impossible.

After being certain of a source of hepatic venous blood, a peripheral venous source was obtained. The femoral vein was usually selected for this Following a priming dose of Bromsulphalein, a constant intravenous infusion of BSP was started in concentrations of 350 mg. per cent A twenty minute period was allowed for stabilization and equilibration of peripheral BSP concentration. Thereafter, samples of hepatic (H) and peripheral (P) blood were withdrawn at 10 minute intervals for a minimum of three periods. This provided data to calculate normothermic splanchnic blood flow The animals were then cooled in cooling blankets through which refrig-

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and this was transient. A one plus cephalin flocculation appeared on the third post-cooling day of one animal. Follow-up at one and three weeks in this animal revealed reversion to normal. Bilirubin, BSP clearance, total proteins and A/G ratios, and prothrombin time remained normal in all experiments. It seems unlikely that a great deal of significance can be attributed to the slight abnormality in cephalin flocculation found in this single instance.

DISCUSSION

The response of the splanchnic blood flow to hypothermia is comparable to the decreases reported in cardiac output, and oxygen consumption by Bigelow[3] The pattern is similar to the decreasing values of pulse blood pressure and urine formation characteristically observed. Taken as a whole, these facts lend support to the tenet that the entire organism undergoes a non-selective type of hypometabolism at reduced body temperature. The one exception to this is probably the skin and superficial muscle where temperatures are considerably lower than that recorded high in the rectum, and metabolism probably near nonfunctional levels. The decreased hydrostatic force, hemoconcentration and vascular stasis are probably factors in accentuating this extensive degree of hypometabolism

The experiments point out the relationship between liver blood flow and Bromsulphalein clearance. Grindley[4] has demonstrated that the ability of the liver to clear Bromsulphalein is dependent upon blood flow. In these experiments, the liver was capable of clearing 1.5 to 2.2 mg. of Bromsulphalein per minute at the normothermic level. At 23°C. the liver was capable of clearing 0 3 to 0 75 mg. of Bromsulphalein. This reflects the marked alteration in rate of function associated with hypothermia. Although hepatic function is subnormal in rate under these circumstances, this does not produce any apparent deleterious effect on qualitative function in the post-cooling period, at least as reflected by the liver function studies performed.

SUMMARY

1. Splanchnic blood flow has been measured under hypothermia. This appears to decrease in arithmetic progression with the decrease in body temperature At 23°C splanchnic blood flow is approximately 22 per cent of the normothermic values

2 Liver function tests performed before and after induced hypothermia failed to demonstrate significant deleterious effects related to cold.

3 Liver function appeared to be quantitatively decreased during the actual phase of hypothermia.

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The fact that the studies on splanchnic blood flow have been initiated under anesthesia may conceivably have altered the absolute accuracy of the values reported herein. The extensive splenic engorgement noted under anesthesia in the dog may trap a considerable volume of the splanchnic blood. However, this condition exists as a relatively constant factor in these experiments. Although only small amounts of anesthetic are employed fol-

Table 2. Splanchnic Blood Flow at Reduced Body Temperature, Detailed Data for Dog 53-332 (ml/min.)

TEMPERATURE, °C	PERIOD	PERIPHERAL BSP (P), MG %	HEPATIC BSP (H), MG %	P-H	QUANTITY BSP INFUSED, MG/MIN.	TIME, MIN.	SPLANCHNIC BLOOD FLOW, ML/MIN.
37*	1	2 46	1.84	0 62	2 24	10	622
	2	2 23	1 63	60	2 19	10	643
	3	2 35	1 73	.62	2 00	10	556
33	4	2 30	1.68	62	1.85	10	506
	5	2 45	1 76	69	1 92	10	477
29	6	2 50	1 80	69	1 56	10	390
	7	2 39	1 70	69	1 50	10	375
26	8	2 71	2 00	71	1 25	10	304
	9	2 64	1 91	73	1 21	10	286
23†	10	3 02	2 22	80	0 76	10	179
	11	2 84	2 10	.74	.71	10	181

*Hematocrit 42.
† Hematocrit 47.

lowing the initial anesthetizing dose, the agent probably remains in effect throughout as it is detoxified extremely slowly under hypothermia. This is suggested further by the frequent observation of delayed recovery of consciousness following rewarming of the animal Thus it appears reasonable to assume that some anesthetic effect is present throughout the experiments, and this makes the relative changes recorded in splanchnic blood flow significant.

Table 3 Evaluation of Liver Function, Composite Data on 8 Experiments

TEST	PRECOOLING RESULT	POST-COOLING RESULTS: 3 DAYS	1 WEEK	3 WEEKS
Prothrombin activity	100%	100%	100%	100%
Total protein	More than 6 5	------	more than 6 5	------
A/G ratio	2 0 or more	------	2 0 or more	------
Cephalin flocculation	Negative	neg(7) 1 (1)	negative	------
Bilirubin	Less than 1 0 mg.	------	less than 1 0 mg	------
BSP clearance	Negative	------	negative	------

Liver Function. Animals in which impairment of liver function was suggested by the tests performed in the precooling period were not used Thus, the precooling liver function studies are all within normal limits. The tests were again repeated in the post-cooling period at intervals previously described A composite picture of the results of these tests appears in Table 3 In only one animal did any evidence of impairment appear,

30 minutes "residual" dye must be determined just before making the next dye injection, and this value subtracted from the subsequent extrapolated zero, but when the interval is thirty minutes or more the residual dye value is so small that it can be safely neglected.

The clearance of RB is determined from the single injection disappearance curve by multiplying the volume of distribution by the slope of the disappearance curve. The formula employed, which has been derived elsewhere,[2] is as follows:

$$\text{Clearance} = 2.303 \frac{V}{t \text{ (min.)}} \log_{10} \frac{\text{Conc. at time } 0}{\text{Conc. at time } t}$$

V is the volume of distribution as determined above.

The constant infusion RB clearance is measured by dividing the rate of dye infusion by the dye concentration at equilibrium. Equilibrium can be reached rapidly by giving a priming dose equal to a ten minute infusion just before starting the infusion.

Measurement of the hepatic blood flow with RB is made by dividing the constant infusion clearance of the dye by the hepatic extraction ratio, the hepatic venous blood being sampled through a catheter. The hepatic extraction ratio is defined as (PA-HV)/PA, where PA is the concentration in a peripheral artery and HV is that in the hepatic vein.

Measurements of the effect of various procedures on the dye clearance were made in some cases by employment of the procedure during a single injection disappearance curve and observing the effect on the slope before, during, and after the procedure. In these experiments it was assumed that the volume of distribution was unchanged by the procedure. In experiments involving hemorrhage single injections were made before and after an acute hemorrhage and both the slope and the measured volume of distribution were used in the clearance calculation.* The effect of hemorrhage on hepatic dye clearance and blood flow was also studied during constant infusions with hepatic venous catheterization

The measurement of extrahepatic dye removal was made in some cases by comparison of arterial and jugular or femoral venous dye concentrations in samples drawn simultaneously during a constant infusion. In addition, the tissues and urine were examined visually for evidence of staining by the dye (which maintains its color at all body *p*H's).

Over 100 dogs, anesthetized with sodium pentobarbital given by the IV route, were used in these experiments.

RESULTS

Figure 1*a* shows a typical disappearance curve of RB from plasma after a single injection Note that the results are plotted on a semilogarithmic scale. The disappearance curve is absolutely linear in this experiment and was found to be so with insignificant deviations in 60 other determinations on 43 dogs. It can be shown that this will occur if, and only if, the clearance is independent of the concentration of dye.

Figure 1*b* illustrates the behavior of Bromsulphalein (BSP) after a single

* Neglecting the volume of distribution makes the dimensions of clearance inappropriate: e g, Bradley's use of PDR with BSP[3] and Laws and Everson's use of log (C_o/C_t) with RB.[4] These measurements are measurements of slope only and cannot be considered as flow.

THE PLASMA CLEARANCE AND VOLUME OF DISTRIBUTION OF ROSE BENGAL*

ARTHUR M. SIMPSON AND LEO A. SAPIRSTEIN

When renal or hepatic clearance of a substance is defined as the ratio of its excretion rate to its plasma concentration, it has the dimensions of flow. Further, it is plain that when clearance so calculated is independent of plasma concentration the determinant of clearance is flow rather than excretory capacity

In the course of a search for a substance which might be used for the estimation of hepatic blood flow (HBF) without hepatic catheterization, we observed that the red dye rose bengal (*tetraiodo-tetrabrom-fluorescein*) had a clearance which was, over a considerable range, independent of its plasma concentration In other words, it could be considered that changes in rose bengal (RB) clearance were directionally similar to changes in HBF. (It will be shown in the discussion that only the direction, and not the exact magnitude of flow changes, can be assessed by measuring the clearance change.)

The independence of RB clearance and plasma concentration further makes possible the estimation of RB clearance from the plasma disappearance curve of this dye after a single injection as well as by the more cumbersome constant infusion techniques. Two other desirable properties of the dye were found, it appeared to have insignificant extrahepatic clearance, and its volume of distribution (determined from the disappearance curve) was found to be equal to that of T-1824.[1] The present communication describes the method of measuring hepatic clearance of the dye by a single injection technique and indicates the effects of various procedures on this clearance It will also be shown that the dye is suitable for the repetitive measurement of blood volume at frequent intervals.

METHODS

RB is conveniently determined in the plasma by direct reading in any photoelectric colorimeter or spectrophotometer at a wave length of 560 mu The method of determination has been described in detail elsewhere.[1] The dye follows Beer's law in both plasma and saline. Doses of 25 mg. in large dogs yield sufficient color in the plasma to permit duplicate determinations after dilution of the plasma 1 to 4 with an accuracy of 1 per cent

The volume of distribution of RB is determined by extrapolation of its plasma concentration curve (which is linear when plotted semilogarithmically against time) to the moment of injection. Samples of blood for plotting the disappearance curve are ordinarily taken at 2, 4, 6, and 8 minutes after the injection is made. Dividing the injected dose by the extrapolated zero time concentration yields the volume of distribution

When repetitive determinations are to be made at intervals of less than

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ml./kg./min. The fact that this value is somewhat smaller than that usually obtained by other authors using a similar method with BSP is presumably related to lower extrahepatic clearance of the RB.*

The effects of epinephrine on the clearance as measured during a single injection were variable. Clearance occasionally rose and then fell, returning to its original value within three minutes after epinephrine administration (Fig. 3*a*). The converse order of rise and fall was observed as often.

Pitressin given IV produced a marked depression of clearance which was greatest during and just after administration of the hormone (Fig. 3*b*).

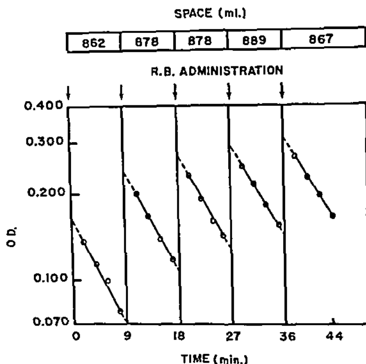


Fig 2 Five consecutive intravenous injections of 15 mg. of RB were given at intervals of 9 minutes After the first injection it is necessary to subtract the residual dye from the extrapolated zero time concentration of the succeeding injection to obtain the true value. The space calculated from each determination is given above Note the relatively small amount of residual dye which must be allowed for, resulting from the rapid elimination of the material, even at such extremely short intervals.

Bilateral carotid occlusion, which presumably causes massive sympathetic discharge, was, surprisingly, without any appreciable effect on the clearance (Fig. 3*c*).

Hemorrhage did not alter the slope of the disappearance curve but displaced it upward (Fig. 4). This signified a smaller volume of distribution, the reduction corresponding well to the size of the hemorrhage, and therefore a smaller clearance. In clearance determinations after hemorrhage by the constant infusion technique, diminished clearance was associated with increased extraction ratio. The HBF, that is to say, fell more (50 per cent)

* In calculating HBF we used an average of the extraction ratios obtained during the period of observation, in contrast to Bradley, who uses the largest A-V difference as the basis for his calculation. Our comparisons are, therefore, with authors who use BSP in a manner similar to us, e g, 42 ± 2 9 (S.E mean) ml./kg./min,[5] 37 ± 2 3 (S.E. mean) ml./kg/min.[6]

injection in a dog. The disappearance of BSP shows a changing clearance with time and unlike RB is dependent on plasma concentration.

The dose of RB injected in the experiment described in Figure 1*a* was 50 mg. The extrapolated zero concentration of dye was 0.068 mg/ml (0.750 OD units/ml). The RB space was calculated to be 737 ml. The T-1824 space in the same dog was 736 ml. In sixteen other dogs in which the two spaces were determined either consecutively or simultaneously, the RB space exceeded the T-1824 space by less than 1 per cent.[1]

The applicability of the dye for repetitive measurements of plasma volume at short intervals is shown in Figure 2 Each line represents the disappearance curve after five single injections at nine minute intervals. The extrapolated zero value in each case was reduced by the "residual" value

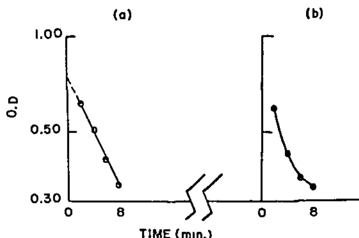


Fig 1 A comparison of a typical plasma disappearance curve after the intravenous injection of 50 mg RB (*a*) and 100 mg BSP (*b*) Note the constant clearance (linearity) over the range of plasma concentrations of RB as contrasted to the changing clearance (curvilinearity) of BSP In addition we can see that whereas extrapolation to zero time for volume of distribution calculation is simple in the RB curve, it is subject to interpretation and thus error in the BSP curve

from the previous injection The five volumes of distribution checked to within ± 2 5 per cent (SD)

A typical calculation of dye clearance from the single injection disappearance curve of Figure 1*a* is illustrated as follows. Using the formula given in the introduction.

$$\text{Plasma clearance} = 2\ 303 \frac{736 \text{ ml}}{8 \text{ min}} \log_{10} \frac{0\ 750}{0\ 340} = 72\ 7 \text{ ml/min}$$

The single injection plasma clearance in 43 consecutive determinations was 5 86 ± 1 77 ml/kg/min A weight basis was found to be suitable for expression of the results. This value was in good agreement with the constant infusion plasma clearance of 5 37 ± 1 07 ml./kg./min. in eighteen dogs.

In ten of the eighteen dogs which had constant infusions, the hepatic extraction ratio for the dye was determined and its mean value was 0 334 ± 0 086. The estimated HBF calculated from the infusion clearance and extraction ratio (plus hematocrit) was found to be 31 5 ± 4.1 (S E mean)

suggests that RB may be a practical substitute for the latter in circumstances where the plasma volume must be measured repeatedly.

The dye appears to offer further advantage over BSP (which in dogs does not make a linear disappearance curve) in that it has lower extrahepatic clearance than the latter dye. Since in the Bradley technique the estimated HBF exceeds true hepatic blood flow by the extent of extrahepatic removal, and since the degree is not known with certainty for BSP, the use of an alternate dye with lower extrahepatic removal would be a desirable improvement in this method of measuring HBF.

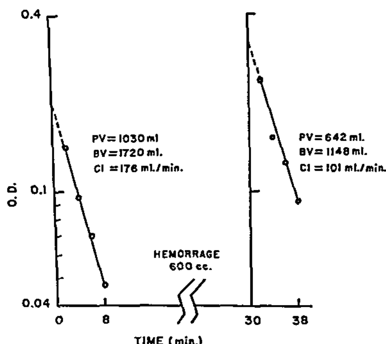


Fig 4 The effect of an acute hemorrhage (30 cc whole blood per kilogram body weight) c
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The effects of epinephrine, Pitressin, carotid occlusion and hemorrhage on RB clearance were qualitatively similar to those reported in the literature for the effects of these substances and procedures on HBF. Unfortunately, the fact that the extraction ratio is not a fixed quantity, but appears to vary (in hemorrhage) in the opposite direction from blood flow, suggests that clearance changes will not fully reflect flow changes, though by the very definition of clearance they do in fact measure "effective" flow in the functional sense.

CONCLUSIONS

The disappearance curve of RB after single intravenous injection of this dye can be used to gain information regarding the plasma volume and dye clearance in units of flow. No extrahepatic removal of this dye has been detected, and the clearance is independent of dye concentration. Although

than did the clearance (30 per cent). It is thus not possible to use clearance changes as a quantitative measure of changes in HBF, but it appears probable that they will afford a directional index of flow changes.

The experiments on extrahepatic removal failed to reveal any detectable arteriovenous dye concentration difference through the areas drained by the jugular or femoral veins. In addition the urine never contained dye, nor was any tissue other than the liver observed to be stained by dye in

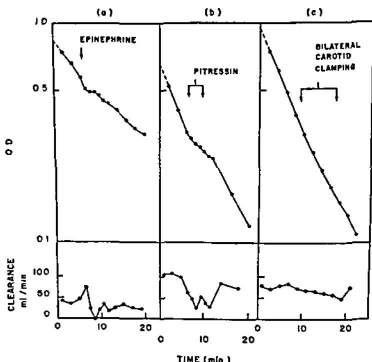


Fig 3 These curves demonstrate the effect of intravenous epinephrine (*a*), Pitressin (20 u at the rate 1 u/20 sec) (*b*), and bilateral carotid clamping (*c*) on the disappearance curve of RB In each case several points on the curve were determined before the procedure under study was instituted, to afford a volume of distribution and normal clearance calculation for comparison with the changes resulting from the procedure. Below the disappearance curve in each case is a linear graph on which is plotted the plasma clearance in ml/min calculated from paired actual points on the curve (0–2, 2–4, 4–6, 6–7, etc).

the doses used. (Doses of 300 mg sometimes resulted in a pink urticaria, but this was localized, and presumably due to drug toxicity.)

DISCUSSION

The use of RB in liver function studies[4,7] has been handicapped by the lack of an agreed unit to describe the elimination of the dye. The employment of the clearance concept remedies this difficulty, and its expression in units of flow makes comparisons from time to time and dog to dog meaningful.

The independence of clearance and concentration permits the single injection disappearance curve to be used for calculation of volume of distribution, and the identity of this value with the T-1824 space in the dog

anesthetized quiet animals which had been previously trained for the experiment.* The Bromsulphalein (BSP) method of Bradley[7] was used without modification for the determination of blood flow. Approximately one hour before beginning the BSP infusion, under local anesthesia and fluoroscopy, a catheter was inserted via the right external jugular vein through the superior and inferior venae cavae into the hepatic vein entering the left lobe of the liver. This vein was used in all experiments. The catheter was anchored at the skin level and its position was fluoroscopically checked before and at the end of the experiment so that there was never any question of its being well into the liver. The patency of the hepatic catheter was maintained by dripping a heparinized saline solution at a rate of 2 to 3 drops per minute through it. Polyethylene tubing was inserted under local anesthesia into the femoral artery. All peripheral blood samples were removed from this catheter. A priming dose of BSP of 2 mg. per kilogram was administered intravenously and this was followed by constant infusion of BSP at a rate of 1.0 to 1.3 mg. per minute through a fine polyethylene

Table 1. Estimated Hepatic Blood Flow in Normal and Arterialized Dogs

	NO. DOGS	WT. KGS.	P_{BSP} MG. %	E_{BSP} %	ΔP MG. %/MIN.	R_{BSP} MG / MIN	HEMATOCRIT	EHBF ML /MIN.	EHBF ML./KG. /MIN.
Normal	20	10 5 ±1.9	1.48	34 2	0 0045	1.22	40	474 ±104	45 5 ± 9.7
Arterialized	10	9 5 ±0 8	1 39	18 0	0 007	1.35	37	1204 ±433	128 0 ±49 0

P_{BSP} = Plasma level of BSP.
E_{BSP} = Hepatic extraction of BSP.
ΔP = Change in level of BSP.
R_{BSP} = Hepatic removal rate
EHBF = Estimated hepatic blood flow.

catheter threaded into a leg vein. BSP was delivered by a constant infusion pump. At 30, 40, 50, and at times 60 minutes, simultaneous blood samples (5 ml.) were drawn from the femoral artery and hepatic vein catheters for plasma BSP determinations.

RESULTS AND DISCUSSION

Determinations of EHBF in 20 normal dogs in this laboratory revealed a mean flow of 45.5 ± 9.7 ml./kg /min. For 13 determinations on 10 arterialized animals the average flow was 128.0 ± 49.0 ml /kg./min. (Table 1).

Values for individual determinations are represented by Figures 1 and 2.

Dog No. 732 (Fig. 3) is an example of a dog with an EHBF determination before arterialization and 7 days following operation. In this instance, the postoperative EHBF is double the preoperative value. The reason for the high normal flow is unexplained. The greatest calculated EHBF was in dog No. 719. Thirty days after arterialization it was found to have a flow of 234 ml./kg./min. (Fig. 4). This dog is presented to demonstrate one of the limitations of the BSP method for estimation of markedly increased hepatic blood flow. In this animal the P_{BSP} (peripheral plasma concentration of

* Subsequent determinations on animals anesthetized with pentobarbital sodium fail to show an appreciable difference.

the clearance value may not vary directly with HBF, it appears to reflect changes in the latter directionally. The rapid disappearance of the dye from the circulation makes it possible to estimate plasma volume as frequently as once every nine minutes.

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HEPATIC BLOOD FLOW STUDIES IN ANIMALS WITH TOTALLY ARTERIALIZED LIVERS*

CLEM RUSS, JOHN HAPPEL, PAUL PRENDERGAST, AND BERNARD FISHER

Using a technique previously described in detail,[1] the dog liver was totally arterialized by the performance of an end-to-side portacaval shunt and the interposition of a previously removed autogenous vein graft between the proximal portal vein and the aorta just below the renal arteries. This operation was done in one stage. Studies on hepatic regeneration in such a preparation[2] would indicate that not only is portal blood not essential for regeneration, but that an increase in blood flow augments regeneration. It not only became necessary to show that such an operative technique actually increases the flow of blood through the liver, but it seems of value to quantitate the magnitude of the change. The purpose of this paper is to report some of our experiences with hepatic blood flow determinations in dogs arterialized for from 3 to 35 days. To our knowledge, this is the first time such information has been presented.

EXPERIMENTAL

The EHBF (estimated hepatic blood flow) was determined in 20 normal and 10 arterialized mongrel dogs of both sexes averaging 10.5 ± 1.9 kg. and 9 5 ± 0 8 kg. in weight, respectively All determinations were made on un-

* From the Department of Surgery, Section on Surgical Research, and the Addison H. Gibson Laboratory, University of Pittsburgh This study was aided by U. S Public Health Grant A-369(C).

met in some of the arterialized animals. On several occasions the concentrations of BSP in the hepatic and peripheral plasma have been equal, or so slightly different that the E_{BSP} was but a few per cent. This, of course, makes a valid calculation of the EHBF impossible under these circumstances. It is to be emphasized that the lack of difference in peripheral and hepatic vein blood was not due to the catheter having become dislodged from the liver.

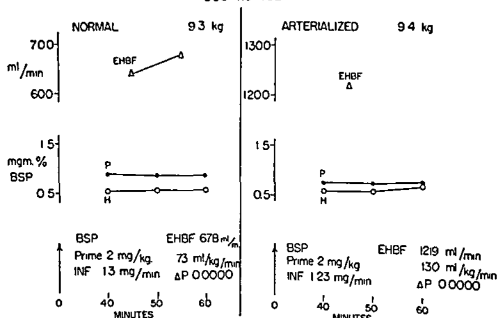


Fig. 3.

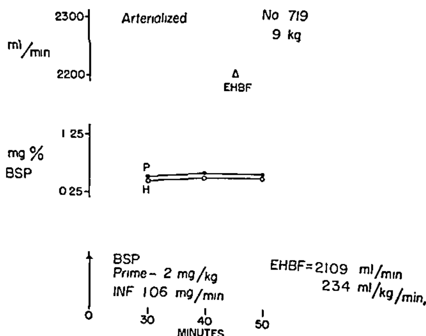


Fig. 4.

BSP) was 0.59 mg. per cent. According to Bradley,[4] the percentage E_{BSP} (hepatic extraction of BSP) when the P_{BSP} is under 1.0 mg. per cent should be greater than 15 per cent for validity. In this instance, the percentage E_{BSP} was 12.7 per cent. When the P_{BSP} is over 1.0 mg. per cent, the percentage E_{BSP} should be greater than 10 per cent. This criterion has not been

ESTIMATED HEPATIC BLOOD FLOW (EHBF) IN TWENTY NORMAL DOGS

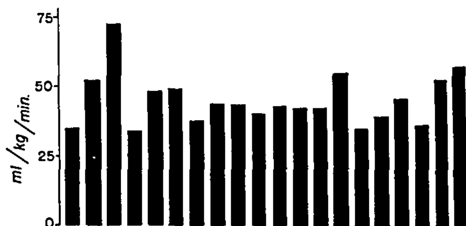


Fig. 1.

HEPATIC BLOOD FLOW (EHBF) AFTER ARTERIALIZATION

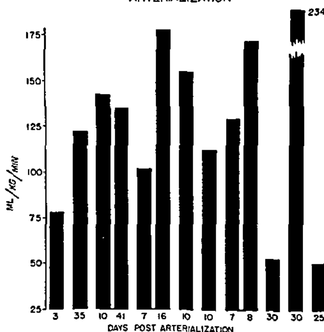


Fig. 2.

Cholesterol can be converted into cholic acid in the course of its metabolism.[6-8] It has been reported that there may be a correlation between hepatic cholesterol production and biliary cholesterol excretion.[9] Other investigators have found that biliary cholesterol level is better related to plasma cholesterol than to hepatic cholesterol.[10]

In earlier experiments in this laboratory, cholic acid and cholesterol were followed in the bile under varied conditions for a two-week period.[11-13] It was therefore of interest to study the biliary excretion of these substances in the alloxan-diabetic rat.

EXPERIMENTAL PROCEDURES

Male Wistar rats averaging 240 grams in initial body weight were maintained on a standard synthetic diet containing: sucrose, 75 per cent; casein, 18 per cent; cod liver oil (Mead Johnson), 3 per cent; salts (Jones 12), 4 per cent; and vitamin and choline supplements. The composition of the diet, with the addition of vitamin K, has been previously described from this laboratory.[14] All the animals were fed *ad libitum*, and were permitted to drink only 0.9 per cent saline. They were housed in separate cages in a room of fairly constant temperature averaging 27° C.

Alloxan in proper dosage has produced permanent diabetes in various species, owing to a partial chemical pancreatectomy. The typical response of the blood sugar following intravenous alloxan is triphasic: (1) hyperglycemia, at two to four hours; (2) hypoglycemia, at six to twelve hours; and (3) a permanent hyperglycemia, following eighteen to twenty-four hours. Prior starvation makes the animals more susceptible to the action of alloxan. Too low a dosage causes a transient hyperglycemia in the third phase from which the animal may recover spontaneously. The hypoglycemia must be counteracted by glucose administration during the first twenty-four hour period to prevent convulsions and death. Too high a dosage causes damage to kidney, liver, and lungs as well as a fatal hypoglycemia. Alloxan is inactivated in blood and tissues within five minutes, so that it should be administered intravenously rather quickly, in appropriate dosage, and in a rather concentrated form.

The animals in this experiment were therefore starved for twenty-four hours prior to the intravenous injection of alloxan monohydrate (Eastman) in a dosage of 45 mg. per kilogram of body weight. For the first twenty-four hours after alloxan administration, the animals were offered a mixture of 5 per cent dextrose in physiologic saline to drink.

Glucose excretion in the urine ranged from one to eight gm. per twenty-four hours, and the blood sugar from 300 to 800 mg. per cent prior to operation. The bile ducts were then [illegible] ther anesthesia according to a method [illegible] Post [illegible] the end of t[illegible] the blood sugars were [illegible] 170 mg. per cent. Total bile output was collected daily [illegible] operation until the termination of the experiment at ten days. [illegible] kept frozen until determinations could be made for choleste[illegible] acid. Cholic acid was dete[illegible] Kopala,[15] [illegible] method d[illegible] and Wilson.[16]

This situation is similar to that reported by Bradley and his group following the administration of a pyrogen to humans Here, likewise, reduced extraction of BSP occurred to such a degree that satisfactory determinations of EHBF were impossible. Attempts by us to avoid the concomitant rise in plasma BSP concentration by changing infusion rates and times of sampling was to no avail.

It has been suggested that in cases of exceptionally high blood flow there is a specific interference with hepatic cellular BSP pickup rather than a reduction in general cellular activity. Since we have found that oxygen consumption and glucose output are not impaired in these animals, we would be inclined to agree with the thought. It is quite probable that in instances of extremely high blood flow the transfer limit for hepatic cells has been reached rapidly and thus the extreme reduction in E_{BSP}.

CONCLUSIONS

1. We have shown that arterialization of the liver increases blood flow through that organ approximately 2 to 3 times the normal,
2. The BSP method for measurement of extremely high blood flows is not adequate, because the extraction rate of BSP is so small that an accurate determination of EHBF is not feasible.

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BILIARY EXCRETION OF CHOLIC ACID AND CHOLESTEROL IN ALLOXAN-DIABETIC RATS*

WILLIAM W. KLATCHKO AND HARRY M. VARS

There may be an elevated plasma cholesterol level as well as elevated ketones in the plasma and urine of untreated diabetic patients In the diabetic animal there is an impairment of the utilization of glucose accompanied by a decreased net synthesis of fatty acids from pyruvate, acetate, and lactate with a concomitant increased production of ketone bodies.[1-3] The diabetic liver is not deficient in fatty acid oxidases or in energizing systems capable of synthesizing cholesterol from two-carbon fragments.[4] As a matter of fact, the incorporation of added acetate into cholesterol is much greater in the liver slices prepared from diabetic rats than in those prepared from normal rats[5]

* From the Harrison Department of Surgical Research, Schools of Medicine, University of Pennsylvania, Philadelphia This work was supported (in part) under contract between the Department of the Army and the University of Pennsylvania

Cholesterol can be converted into cholic acid in the course of its metabolism.[6-8] It has been reported that there may be a correlation between hepatic cholesterol production and biliary cholesterol excretion.[9] Other investigators have found that biliary cholesterol level is better related to plasma cholesterol than to hepatic cholesterol.[10]

In earlier experiments in this laboratory, cholic acid and cholesterol were followed in the bile under varied conditions for a two-week period.[11-13] It was therefore of interest to study the biliary excretion of these substances in the alloxan-diabetic rat.

EXPERIMENTAL PROCEDURES

Male Wistar rats averaging 240 grams in initial body weight were maintained on a standard synthetic diet containing: sucrose, 75 per cent; casein, 18 per cent; cod liver oil (Mead Johnson), 3 per cent; salts (Jones 12), 4 per cent; and vitamin and choline supplements. The composition of the diet, with the addition of vitamin K, has been previously described from this laboratory.[14] All the animals were fed *ad libitum,* and were permitted to drink only 0.9 per cent saline. They were housed in separate cages in a room of fairly constant temperature averaging 27° C.

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The animals in this experiment were therefore starved for twenty-four hours prior to the [illegible] of alloxan monohydrate (Eastman) i[illegible] ody weight. For the first twenty-four h[illegible] animals were offered a mixture of 5 per cent dextrose in physiologic saline to drink.

Glucose excretion in the urine ranged from one to eight gm. per twenty-four hours, and the blood sugar from 300 to 800 mg. per cent prior to operation. The bile ducts were then cannulated under ether anesthesia according to a method previously devised in this laboratory.[11] Post-cannulation, toward the end of the experimental period, the blood sugars were all higher than 170 mg. per cent. Total bile output was collected daily from the time of operation until the termination of the experiment at ten days The bile was kept frozen until determinations could be made for cholesterol and cholic acid. Cholic acid was determined by the method of Irvin, Johnston, and Kopala,[15] and cholesterol by a modification of the Schoenheimer and Sperry method described by Foldes and Wilson.[16]

RESULTS AND DISCUSSION

In Figure 1 are plotted the average daily values obtained for biliary excretion, and food and saline intake of alloxan-diabetic rats and their controls following ductal cannulation. It can be seen that it takes from three to four days following operation for these experimental values to approach a steady state. Accordingly, we have chosen the period of days four through nine as reflecting a better approximation of the functional capacity of the two

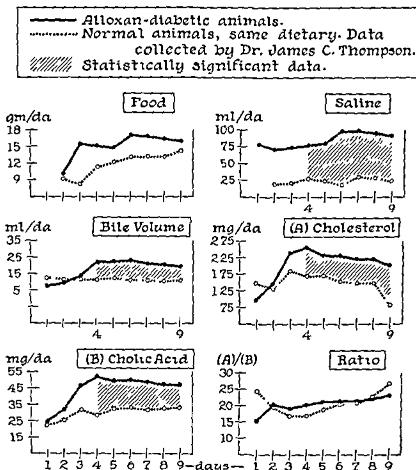


Fig 1 Daily average values of biliary excretion and food and saline intake of alloxan-diabetic rats and controls following ductal cannulation.

groups of animals. Table 1 summarizes the mean values for the whole six-day collection period (days four through nine) with their standard deviations, and presents a statistical analysis of the data.

There was an increased bile volume which was probably related to polydypsia in the alloxan-diabetic group of animals. Biliary excretion of cholesterol and cholic :
related to food intake.
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fistula rat, more cholesterol and cholate are produced in the liver and in turn excreted in the bile.

SUMMARY

1. Alloxan-diabetic animals were prepared and subjected to cannulation of the common bile duct. Total bile output was followed for ten days, the fourth through ninth days' values for cholate and cholesterol were then compared to controls fed the same dietary.

2. There was an increased bile volume, probably related to polydypsia in the alloxan-diabetic animals.

Table 1. Daily Biliary Excretion, and Food Intake of Normal and Alloxan-Diabetic Rats for the Fourth through the Ninth Day after Bile Duct Cannulation

ANIMALS	FOOD INTAKE	BILE EXCRETED: VOLUME	CHOLIC ACID (A)	CHOLESTEROL (B)	RATIO
No.	Gm	ml.	mg.	mg	(A)/(B)
Normal (12)	13 3 ± 1 91	11 1 ± 1.65	31.7 ± 3 2	1.48 ± 0.13	21 6 ± 2 6
Diabetic (8)	15 6 ± 3 7	21.1 ± 7 55	48 8 ± 4.64	2.25 ± 0.395	22 2 ± 4 3
Normal $S\bar{x}$	0 552	0 476	0 92	0 37	0 745
Diabetic $S\bar{x}$	1.28	2 66	1 9	0 162	1.51
t (n = 18)	1 8555	4 4843	9 243	6 311	0 412

The data give mean values with their standard deviation
$S\bar{x}$ — represents the standard error of the mean.
t — ratio of mean/standard error of the mean.

3. There was a highly significant elevation in biliary cholesterol and cholate, probably related to the metabolic imbalance induced by alloxan-diabetes in the biliary-fistula rat rather than to food intake.

4. This can be interpreted as indicating that more cholesterol and cholate are produced in the liver and excreted in the bile under these conditions.

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BLOOD VOLUME DEFICITS IN PANCREATITIS*

LUTHER M. KEITH, JR., AND ROBERT N. WATMAN

Pancreatitis is currently recognized as a common disease of uncertain or multiple etiologies for which treatment in the acute phase is fundamentally supportive. Consequently, mortality remains significant and the mechanism of death is controversial. Therefore, any information resulting in improvement of treatment or reduction in mortality from pancreatitis would seem to be a valid contribution. Experimental studies and a review of cases succumbing to pancreatitis in this clinic have shown that shock is prominent in [illegible]d [illegible]d- [illegible]d and are reported in an effort to evaluate the role of clinical deficits in this disease. Studies of this type applied to other clinical entities such as acute intestinal obstruction and carcinomas of the gastro-intestinal tract have yielded valuable information in recent years.

Actual blood volume determinations were performed by the minimal dose R.I.H.S.A. method in 14 cases of acute pancreatitis, 11 patients with chronic pancreatitis† and 6 subjects with acute recurrent disease. These were compared to theoretical normal values determined by Gregerson's method[1] and corrected by Randall's factors.[2] The mean and maximum volume deficits and average percentile deficits of whole blood volume, plasma volume and circulating red cell mass were calculated for each of the groups studied.

Impressive initial deficits of all the components of the blood volume were found in both acute and chronic pancreatitis. These results emphasize the

importance of this facet of the disease and the value of blood volume determinations which allow quantitative replacement of deficits (Fig. 1).

The findings in patients with each type of pancreatitis will be presented separately.

ACUTE PANCREATITIS

In 14 cases of confirmed acute pancreatitis, the mean whole blood volume deficit was 1806 cc.; the average plasma volume deficit was 1260 cc. and the average deficiency in red cell mass was 874 cc. The maximum whole blood deficit for the group was 2510 cc., maximum plasma volume deficit 2136 cc. and maximum red cell mass deficiency was 1375 cc. (Fig. 2).

The average percentile deficit in each component of the blood volume was calculated and found to be 30.2 per cent of whole blood volume, 27.4 per cent of plasma volume and 33.1 per cent of normal red cell mass (Fig. 3).

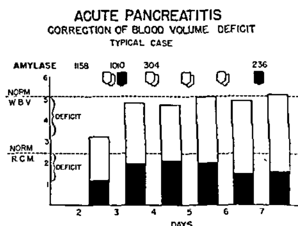


Fig 1. Replenishment of initial blood volume deficit found in a patient with acute pancreatitis Each large flask = 500 cc. whole blood. Each small flask = 100 cc. concentrated human serum albumin Replacement would have been more ideal with greater proportion of whole blood. W.B V. = whole blood volume, R.C M = red cell mass. (Adapted from New England Journal of Medicine, 251:498, 1954)

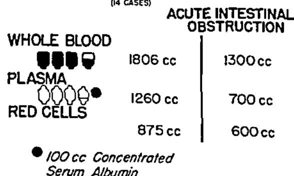


Fig 2. Average initial whole blood, plasma and red cell mass deficits in 14 cases of acute pancreatitis compared with average corresponding depletions in 27 cases of acute intestinal obstruction. Initial requirements for replacement indicated by bottles of whole blood and *serum albumin*

CHRONIC PANCREATITIS

Similar depletions were found in 11 patients with chronic pancreatitis. The mean deficits were 1572 cc. in whole blood volume, 791 cc. in plasma volume and 831 cc. in red cell mass. Corresponding maxima were 3749 cc., 1882 cc and 1877 cc. (Fig. 4).

The average percentile deficits for the group with chronic pancreatitis

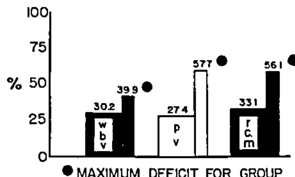


Fig 3 Mean and maximum percentage deficiencies in each component of blood volume in 14 patients with acute pancreatitis. w b v = whole blood volume; p v. = plasma volume, r c m. = red cell mass

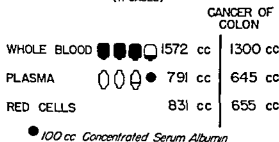


Fig 4 Average initial whole blood, plasma and red cell deficiencies in 11 cases of chronic pancreatitis compared to average corresponding values in 58 patients with malignancies of colon Bottles of whole blood and *serum albumin* represent initial requirement for replacement.

were 27.3 per cent of whole blood volume, 25 per cent of plasma volume and 32.1 per cent of circulating red cell mass (Fig. 5).

RECURRENT PANCREATITIS

Compared with the cases of acute and chronic pancreatitis, a group of 6 cases of recurrent pancreatitis showed surprisingly small depletions of whole blood volume and plasma volume. Deficits of red cell mass, however,

were entirely comparable. Average deficits were 757 cc. of whole blood volume, 200 cc. of plasma volume and 618 cc. in the red cell mass. Corresponding percentile deficits were 9 6 per cent of whole blood volume, 8.7 per cent of plasma volume and 22 per cent of red cell mass.

DISCUSSION

The ubiquitous deficits in all components of the blood volume indicate the prominence of these factors in the morbidity and mortality resulting from the various forms of pancreatitis. These data emphasize the importance

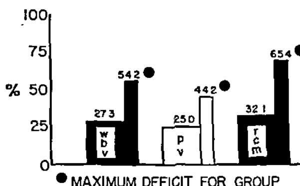


FIG. 5. Mean and maximum percentage deficits in each component of blood volume in 11 cases of chronic pancreatitis. w b v. = whole blood volume, p v. = plasma volume, r c m. = red cell mass.

of evaluating and quantitatively replenishing the blood volume deficits in a disease dependent on supportive therapy. The frequency of similar deficiencies and the presence of "chronic shock" accompanying many gastrointestinal lesions has been stressed. It is therefore interesting to note that the blood volume deficiencies in acute pancreatitis exceeded those found in patients with acute intestinal obstruction while the deficits found in chronic pancreatitis were in excess of those found in patients with cancers of the colon [3]

The investigations of Elliott et al.[4] and Keith[5] would suggest that this blood volume depletion in acute pancreatitis is partially due to transudation of fluid around the pancreas and into the peritoneal cavity. Other mechanisms of fluid loss have been suggested.

The explanation of blood volume deficits in chronic pancreatitis is less apparent. The nutritional aspect is probably most important, since these patients soon discover that eating often precipitates painful attacks. Furthermore, concomitant cholangitis may also impair hepatic synthesis of plasma proteins. The smaller plasma volume deficits observed in recurrent pancreatitis may represent compensation for anemia since the disease is of shorter duration and liver disease occurs less often. Increase in plasma volume is recognized in other conditions producing anemia.

However this may be, these findings have been useful in supplementing the treatment of pancreatitis by quantitative replacement of these surpris-

ingly large deficits in all components of the blood volume. Repeated blood volume determinations during replacement therapy have shown that the underlying mechanism of depletion remains operative. The restitution of adequate blood volume, therefore, frequently requires more whole blood and serum than the initially measured deficit indicates. Since recognition of these factors, the utilization of blood volume determinations and the replacement of deficits has resulted in accelerated recoveries and significant decrease in the mortality from pancreatitis in this clinic.

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THE MECHANISM OF BENEFIT DERIVED FROM CONCENTRATED HUMAN SERUM ALBUMIN IN EXPERIMENTAL ACUTE PANCREATITIS*

DAN W. ELLIOTT

Human serum albumin has been suggested as the source of a concentrated trypsin inhibitor (antifibrinolysin), which should be of specific therapeutic value in acute hemorrhagic pancreatitis, and observations have been presented indicating that albumin was of benefit in a series of patients.[1] To provide objective laboratory confirmation, studies have been undertaken to evaluate the influence of albumin on pancreatitis induced in dogs, and particularly, to determine whether or not inhibition of trypsin is the significant mechanism of benefit. Blood volume determinations have also been made since concentrated albumin has important osmotic effects in the expansion of plasma volume and the treatment of shock

The presence of a trypsin inhibitor, antifibrinolysin, in serum prevents a satisfactory direct assay of pancreatic [illegible]

[illegible] proteolytic activity (activated plasminogen) rises during the acute phase of pancreatitis induced in dogs.[2] These same effects are seen following the

* From the Department of Surgery and the Surgical Laboratories, Ohio State University College of Medicine, Columbus This study was aided in part by a grant from the National Institutes of Health, Bethesda, Maryland.

intravenous administration of trypsin,[3] and the toxic effects of intravascular trypsin in producing thrombi, shock and death are well known.

A very potent specific inhibitor of trypsin, extracted from soybeans, has been administered by several investigators in different laboratories to animals in which acute pancreatitis has been induced, with little or no effect on mortality.[2,4,5] In addition, purified crystalline trypsin of pancreatic origin has been well tolerated if given slowly, when administered intravenously in large enough quantities to obtain rather marked anticoagulant effects.[3] These latter observations would seem to minimize the importance of trypsin as a lethal toxin during pancreatitis.

The administration of trypsin intravenously produces, in addition to a fall in antifibrinolysin titer, profound temporary reduction in fibrinogen levels.[3] In the studies reported here, antifibrinolysin titers and fibrinogen levels were followed serially, and significant decrements were taken as indirect evidence for the entry of trypsin into the blood stream. Changes in these two plasma factors were compared following the induction of pancreatitis in a series of dogs, and following the administration of trypsin in a quantity which was [illegible] treated with a potent [illegible] with dextran. Effects [illegible] ie, and mortality were examined.

METHODS

Mongrel dogs of both sexes were used which had been observed for a period of at least two weeks in the laboratory. They were selected for weights as near an average of 12 kilos as possible, and had normal findings several days before the experiment in amylase, lipase, hematocrit, fibrinogen, and antifibrinolysin titer run by methods previously described.[6] Blood volumes were determined by dilution using radioactive iodinated serum albumin, with the first determination made following the induction of anesthesia.

Pancreatitis was induced under sodium pentobarbital anesthesia by the retrograde injection of bile into the accessory pancreatic duct, which was approached transduodenally, and a blunt cannula was anchored in place by ligatures. Bile aspirated from the gallbladder was injected under manometric control, in an effort to standardize injection pressures from animal to animal. Regurgitation of bile back into the duodenum was prevented by occluding the main pancreatic duct just outside the ampulla of Vater with a temporary ligature. Exactly 4 cc. of bile were introduced into the pancreas (if the dog weighed more than 15 kilos 6 cc. of bile were used), following which all occluding ligatures were removed and the duodenum and abdominal wall closed.

Interstitial spread of the bile could be seen on the surface of the pancreas almost immediately, usually with fairly good distribution over the entire gland. Bile-stained edema with a few scattered points of focal hemorrhage rapidly followed, and the surface could be seen to literally weep ascitic fluid before the abdomen could be closed.

One hour following the induction of pancreatitis a second blood volume was determined, following which the experimental treatment was given. Blood samples f[illegible] intervals, and a [illegible] following pancre[illegible] was repeated following this determination

and the dog removed from the table. Blood studies were obtained in most dogs at twelve hours after the onset of pancreatitis. At 24 hours, if the dog was still alive, blood tests were repeated, following which treatment was given for the third and last time. Blood withdrawn for sampling did not exceed 10 per cent of the dog's measured blood volume, or 100 cc. To limit this loss, amylase and lipase levels were obtained at eight or twelve and 24 hours only following operation

Because initial amylase levels were so varied and frequently as high as 1000 units, subsequent values had to be interpreted in each animal in relation to its preoperative level. However, in every dog in which an effective pancreatitis is presumed in these data, amylase elevations reached at least double initial values within twelve hours, and were usually three to four times these levels.

All dogs dying in the course of the experimental work were autopsied as soon after death as possible Large quantities of thin serous blood-tinged ascitic fluid were invariably encountered, and this was found to contain three or four Armour units of trypsin per cc as well as large quantities of lipase. The pancreas was enormously swollen and edematous, with hemorrhagic striae appearing in the lobules. Patches of fat necrosis were seen about the margins and usually were scattered in the mesentery, only occasionally reaching the perirenal fat and mediastinum.

Survivors had laparotomy performed at ten days to two weeks following pancreatitis, at which time clinical recovery seemed complete. Fat necrosis and ascites were no longer evident, but the pancreas showed great thickening with a woody indurated texture, and was shrunken in size, with increased lobulations. Microscopic sections showed loss of the normal architecture, fibrosis, some residual hemorrhage, and round cell infiltration. It appeared that a diffuse pancreatitis had occurred.

As a control for the anesthesia and the operative procedure necessary to the induction of pancreatitis, a group of dogs were subjected to exactly the same laparotomy and series of blood tests without the actual injection of bile. To three dogs subjected to this procedure purified crystalline trypsin was administered intravenously, dissolved in saline, at 1000 units per cc, and given at 2000 units per minute, to a total dose of 20,000 units per kilo of body weight

Six dogs in which pancreatitis had been induced were not treated. Three additional dogs received trypsin inhibitor extracted from soybeans, 125 mg. per kilo, at one hour and again at eight hours following pancreatitis. Each mg of this preparation was roughly capable of inhibiting one mg of Armour's Tryptar by antifibrinolysin titer *

Dextran commercially prepared as a 6 per cent solution in normal saline was administered to six dogs at the usual intervals of one, eight, and 24 hours following pancreatitis, at a dose of 20 cc per kilo, given in about 90 minutes.

Concentrated human serum albumin was given in several different ranges of dosage, but at the same intervals, and was administered as rapidly as possible. This human albumin seemed to be remarkably well tolerated by the dogs. Since it had been heated to 60° C. for ten hours to destroy hepatitis virus, some denaturization of the specificity of the proteins may have

* Prepared for this study by Dr Robert O Moore of the Department of Agricultural Biochemistry of the Ohio State University.

occurred. Six dogs have been followed for six months or longer following its administration, and have shown no ill effects. This is the same albumin readily available for human use through the American Red Cross, which furnished a supply that made this study possible.

RESULTS WITH DISCUSSION

The changes in antifibrinolysin titer found in each treatment group are presented in Table 1. In all the tables, averages summarize for each group

*Table 1. Percentage Change in Serum Antifibrinolysin Titer**

GROUP	NO. OF DOGS	OPERATIVE PROCEDURE	TREATMENT	HOURS FOLLOWING LAPAROTOMY OR PANCREATITIS					
				2	4	6	8	12	24
1	4	Laparotomy only	None	−16%	−12%	.	−14%	−12%	−8%
2	3	Laparotomy only	Trypsin IV 20,000 u./kg.	−33%	−39%	−56%	−47%	−33%	+17%
3	6	Pancreatitis	None	−19%	−21%	−31%	−24%	−5%	Dead
4	3	Pancreatitis	Trypsin inhibitor	−5%	−13%		−12%	−0%	Dead
5	6	Pancreatitis	Dextran 20 cc./kg.	−6%	−11%		−28%	−40%	−26%
6	4	Pancreatitis	Albumin 3 cc./kg	−28%	−41%		−43%	−30%	Dead
7	15	Pancreatitis	Albumin 5 to 7 cc./kg						
		Died:		−28%	−47%	−32%	−28%	−32%	Dead
		Survived:		−14%	−22%	−25%	−30%	−42%	−30%

* Average figures for each treatment group of the percentage change from baseline in each animal

the percentage change from preoperative baseline found individually in each dog.

The simple laparotomy necessary to the induction of pancreatitis did not appear to disturb the antifibrinolysin titer to a significant degree (group 1), but a fall in titer of more than 50 per cent uniformly occurred following intravenous trypsin (group 2), as expected.[3] These three dogs survived the intravenous trypsin with no apparent ill effects. However, six dogs with untreated pancreatitis (group 3) all died in from ten to seventeen hours, but showed roughly one-half as great a fall in antifibrinolysin titer as followed intravenous trypsin, although enough fall took place to indicate that some trypsin entered the blood stream during the experimental pancreatitis induced.

The administration of soybean trypsin inhibitor in the amounts used following pancreatitis (group 4) apparently inhibited any liberated trypsin sufficiently well to prevent a significant fall in antifibrinolysin titer, but did not yield any survivors. The amounts of inhibitor at hand prevented its use in more than three animals.

Following the administration of both dextran and albumin generally greater and more prolonged falls in titer were seen than following untreated pancreatitis (groups 5, 6, and 7). In the case of albumin this was unex-

and the dog removed from the table. Blood studies were obtained in most dogs at twelve hours after the onset of pancreatitis. At 24 hours, if the dog was still alive, blood tests were repeated, following which treatment was given for the third and last time. Blood withdrawn for sampling did not exceed 10 per cent of the dog's measured blood volume, or 100 cc. To limit this loss, amylase and lipase levels were obtained at eight or twelve and 24 hours only following operation

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*Table 3. Percentage Change in Plasma Volume and Red Cell Mass; Peak Lipase Values**

GROUP	NO OF DOGS	OPERATIVE PROCEDURE	TREATMENT	HOURS FOLLOWING LAPAROTOMY OR PANCREATITIS 8 PLASMA	8 RBC	12 PLASMA	12 RBC	24 PLASMA	24 RBC	LIPASE AVERAGE OF THE PEAK VALUES IN EACH DOG
1	4	Laparotomy only	None	−18%	+15%	−16%	−9%	−17%	−14%	1 2
2	3	Laparotomy only	Trypsin IV 20,000 u /kg	+3%	+8%	−17%	−10%	+2%	−15%	0.7
3	6	Pancreatitis	None	−45%	−4%	−43%	+2%	Dead		9.5
4	3	Pancreatitis	Trypsin inhibitor	−31%	−11%	−45%	−13%	Dead		5.7
5	6	Pancreatitis	Dextran 20 cc./kg.	−15%	−2%	+1%	+1%	+6%	−3%	6 6
6	4	Pancreatitis	Albumin 3 cc /kg	−39%	−12%	−21%	−4%	−33%	−8%	6 5
7	15	Pancreatitis	Albumin 5 to 7 cc /kg.							
	5	Died:		−36%	−14%	−33%	−5%	Dead		6 6
	10	Survived:		−6%	+7%	+2%	−10%	−3%	−5%	6.0

* Averages summarize for each treatment group the percentage change from the initial in each animal.

pected, since if concentrated antifibrinolysin had been added with the albumin some rise in titer following its administration might have been expected Both dextran and albumin in a number of lots were then tested for capacity to inhibit trypsin in vitro by several methods, and neither showed any active trypsin inhibition whatsoever. It seems probable that the original antifibrinolysin content of the albumin has been destroyed in its processing.

The changes in fibrinogen levels are presented in Table 2. Significant changes occurred in groups where there was a fall in antifibrinolysin titer, which seems to confirm that these changes are due at least in part to liberated trypsin. The administration of soybean trypsin inhibitor apparently

*Table 2. Percentage Change in Plasma Fibrinogen Level**

				HOURS FOLLOWING LAPAROTOMY OR PANCREATITIS			
GROUP	NO. OF DOGS	OPERATIVE PROCEDURE	TREATMENT	4	8	12	24
1	4	Laparotomy only	None	−11%	−9%	+7%	+58%
2	3	Laparotomy only	Trypsin IV 20,000 u./kg.	−63%	−62%	−59%	+3%
3	6	Pancreatitis	None	−11%	−22%	0%	Dead
4	3	Pancreatitis	Trypsin inhibitor	−9%	−10%	−1%	Dead
5	6	Pancreatitis	Dextran 20 cc./kg	−41%	−33%	−54%	−7%
6	4	Pancreatitis	Albumin 3 cc./kg	−29%	−27%	−27%	Dead
7	15	Pancreatitis	Albumin 5 to 7 cc./kg				
		Died		−15%	−15%	+8%	Dead
		Survived		−30%	−23%	−22%	+10%

* Average figures for each treatment group of the percentage change from baseline in each animal.

prevented any significant decrease in fibrinogen level following pancreatitis, although fibrinogen changes in untreated dogs were modest (group 4 compared with group 3).

The changes in plasma volume and in red cell mass are presented in Table 3, together with peak lipase values. The most striking finding appeared following untreated pancreatitis, where there was a uniform and profound loss of plasma volume averaging 45 per cent (group 3). This was not observed following trypsin or simple laparotomy (groups 2 and 1). The treatment of pancreatitis with trypsin inhibitor did not alter this plasma loss (group 4), and all the untreated or inhibitor-treated dogs died.

Where insufficient albumin was given to prevent plasma loss, no survivals were obtained (group 6), but as increasing doses were used, survivals appeared (group 7). Of the five dogs in group 7 which died, four received only 5 cc. of albumin per kilo, and the fifth received 6 cc. per kilo at each administration Of the ten survivals following albumin, nine received 6 or 7 cc per kilo. As the experiment progressed it appeared that survivals of pancreatitis could be produced at will if enough albumin were used. Apparently sufficient dextran was given to compensate for this plasma loss (group 5), and four out of six treated dogs survived the pancreatitis. The

STUDIES ON PANCREATITIS*

IV. The Pathogenesis of Bile Pancreatitis

ALAN THAL

Following Opie's demonstration of the common channel effect produced by an ampullary calculus the important role of bile in the causation of acute hemorrhagic pancreatitis has received wide acceptance. The hiatus in this theory lies in the demonstration by Mann and Giordano[1] that bile pancreatitis can only be produced experimentally by injection at the tremendous pressures necessary to cause ductal rupture.[2] In spite of this objection it must be conceded that bile on gaining access to the pancreatic interstitium rapidly produces the classic lesions of hemorrhagic pancreatic necrosis. The present study was carried out to elucidate the fundamental mechanism whereby bile produces a lesion so closely resembling the naturally occurring disease. It will be shown that bile when introduced into the interstitial tissues rapidly produces extensive and prolonged stasis of blood flow and that this local circulatory standstill is of such magnitude as to suggest that it is the primary and dominant factor in the pathogenesis of the resulting pancreatic necrosis.

MATERIALS AND METHODS

Animals. Adult 2 to 3 kg albino rabbits and mongrel 10 to 12 kg. dogs of both sexes were used.

Bile. The bile used was freshly procured from the gallbladders of rabbits or dogs and used in the same species.

Injection Apparatus. The method of cannulation and injection of the pancreatic duct of the rabbit have been previously described.[3] The pressure bottle and reservoir are illustrated in Fig. 1.

Transillumination Apparatus This consists essentially of a powerful light source (100 watt) and a Lucite cylinder one inch in diameter bent in its terminal portion to reflect light at a right angle. A Lucite support was used to hold the pancreas as illustrated in Figure 1.

Preparation of the Pancreas. The pancreas of the rabbit was exposed through a midline incision and mobilized by division of the ligament of Treitz. The pancreatic duct was then cannulated and several ligatures passed around the duodenum at multiple points for traction. The duodenum was then stretched out over the Lucite plate in such a manner as to display the thinner portions of the pancreatic lobules over the light source. Observations of blood flow were made using a binocular dissecting microscope. A constant drip of Ringer's gelatin was maintained to prevent desiccation during the long periods of observation.

EXPERIMENTAL

The Factor of Ductal Rupture. In preliminary experiments it was regularly shown in both dogs and rabbits that bile introduced at low pressures (20 to 25 mm. Hg) into the obstructed ductal system is largely innocuous

* From the Department of Surgery, University of Minnesota, Minneapolis. This study was supported by a grant (H-1902) from the United States Public Health Service.

use of larger quantities of dextran was not explored. No differences in amylase or lipase elevations (Table 3) or in blood pressure changes appeared to account for the survivals obtained.

If all the dogs receiving albumin are divided into two groups according to death or survival, a significant difference appears between them in the plasma volume changes (groups 6 and 7, Table 3). In the eight-hour determinations an average plasma loss of 38 per cent appears in the dogs which died, and the actual values ranged from −30 per cent to −46 per cent Among the dogs which lived the average change was small, and values ranged from −22 per cent to +20 per cent. The measured losses in plasma volume which accompanied this experimental pancreatitis seemed entirely consistent with the degree of ascites and pancreatic edema observed. Survival of the pancreatitis appeared more related to therapeutic replacement of plasma volume than to any differences in trypsin activity or inhibition

In summary, an experimental pancreatitis was induced in a series of dogs which was uniformly and rapidly fatal if untreated Using changes in serum antifibrinolysin titer and plasma fibrinogen as indices of systemic tryptic activity, it appeared that some trypsin was liberated during acute pancreatitis, but less than quantities well tolerated by the dogs. Marked plasma volume loss during pancreatitis was observed, and survivors of the pancreatitis were obtained if this plasma loss was compensated by albumin. Four of six dogs with plasma volume loss compensated by dextran also survived pancreatitis

Concentrated albumin now generally used has no capacity to inhibit trypsin, but is beneficial in acute pancreatitis for its osmotic effects in expanding plasma volume.

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anything more than sluggish oozing of small quantities of dark blood. This finding suggested that extensive alterations in blood flow occurred during the course of bile pancreatitis. Accordingly, the effect of bile on blood vessels was studied using precisely the same techniques as were detailed in a recent investigation of the vascular effects of staphylococcal toxin.[4]

To summarize these results it was found that injection of bile into the interstitial tissues between medium sized mesenteric blood vessels produced variable degrees of segmental spasm. By far the most profound effect, however, was seen when small amounts of bile were injected into the mesenteric capillary bed, transilluminated for detailed observation. Within 10 minutes the normally vigorous capillary flow became sluggish in areas of bile diffusion. Initially in the larger vessels the peripheral flow would slow and cease while central flow continued sluggishly. By 15 minutes, however, all capillary flow had ceased and the engorged capillary vessels stood out as if petrified. Further studies revealed that identical vascular phenomena could be reproduced with 3 to 8 per cent solutions of sodium taurocholate, making it likely that the bile salts are the probable vasotoxic substances implicated here.

It should be noted that these effects of bile on the vascular system were regularly observed in tissues remote from the pancreas, thus obviating the factor of tryptic action.

Stasis of the Pancreatic Circulation during the Development of Bile Pancreatitis. The above findings suggested that the primary effect of bile in experimental pancreatitis was to produce suppression of pancreatic blood flow by virtue of its direct effect on blood vessels. To test this hypothesis further the following experiment was carried out on 10 rabbits.

Under Nembutal anesthesia the duodenal loop was delivered through a midline incision and the pancreatic duct cannulated and connected to the bile reservoir and injection apparatus. The duodenal loop was then arranged over the illuminator system as illustrated in Figure 1 so as to present a relatively thin pancreatic lobule in a single high power field. With a satisfactory preparation the pulsating arterioles and small veins and brisk capillary flow could readily be seen. After observing the normal for about 10 minutes, 0 5 cc. of bile was introduced into the pancreatic duct at 20 mm. Hg. After a variable period of time the normally inconspicuous pancreatic ductules were revealed by an advancing column of yellow bile. Continual observation for as long as 2 hours after the bile had reached the finer ramifications of the ductules revealed no alteration in the normally brisk blood flow. Injection of bile at duct rupturing pressure (200 to 300 mm. Hg), however, produced a rapid change in lobular circulation. Within 5 minutes after bile was seen to burst out of the ductules into the interstitial tissue the capillary circulation became sluggish and the cells closely packed. Inspection of veins and arteries supplying the lobule showed areas of segmental spasm of variable degree. This was especially marked in the veins, where areas of spastic occlusion alternated with areas of extreme venous distention By 10 to 15 minutes lobular circulation had virtually ceased and the stagnant columns of blood in the vessels had taken on the dark hue of extreme deoxygenation. By 20 minutes punctate ruptures of capillary walls became evident and subsequently extravasation of red cells also occurred from venules, giving the pancreas a hemorrhagic appearance. During the entire period of observation, which in general lasted 3 hours, there was no

and produces only the morphologic changes resulting from simple ductal obstruction. On the other hand, severe and rapidly fatal hemorrhagic pancreatic necrosis occurred consistently when bile was forcibly injected at pressures sufficient to produce ductal rupture (250 to 350 mm. Hg).

The Effect of Direct Interstitial Injection of Bile in the Dog Pancreas. To find out whether the action of rupturing the ducts itself is a prerequisite in the causation of bile pancreatitis or whether the essential factor is the presence of bile in the interstitial tissue, the following experiments were carried out on dogs.

Under Nembutal anesthesia, using aseptic precautions, 2 cc. of bile was withdrawn from the gallbladder. Then, using a syringe and No. 27 gauge

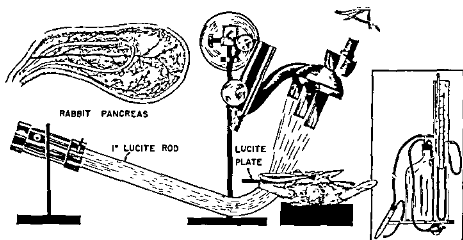


Fig. 1. Showing transilluminator system and arrangement of pancreas for observation of the pancreatic blood flow in the anesthetized rabbit Inset· Constant pressure system for injection of pancreatic duct

hypodermic needle, 0 1 to 0.2 cc. of bile was carefully injected into the interstitial tissue between a few pancreatic lobules at multiple sites By exercising care this could be accomplished without trauma to blood vessels or parenchyma. As a control the opposite tail of the pancreas received only saline injections. All injection sites were carefully diagrammed before closing the laparotomy incisions It should be noted that the pancreatic ducts of Santorini and Wirsung were not obstructed during this experiment. When sacrificed 24 hours later the areas where the bile had been injected regularly showed marked induration and extensive coagulation necrosis with hemorrhage and occasional areas of adjacent fat necrosis. This contrasted sharply with the normal appearance of the uninjected pancreas and the areas where saline alone had been injected. It seems apparent then that the presence of bile within the pancreatic interstitium is the mandatory factor in producing bile pancreatitis and that ductal rupture is merely incidental in attaining this end.

The Vascular Effects of Bile. During the course of another experiment in dogs it was incidentally found that incision into the pancreas of animals in which bile pancreatitis had been induced a few hours previously failed to produce the profuse bright bleeding which occurs when the normal pancreas is traumatized. Indeed, large areas of pancreas could be incised without

EXPERIMENTAL HEMORRHAGIC PANCREATITIS*

I. A Method of Production of Non-infected Lethal Hemorrhagic Pancreatitis

M. HARA, J. C. BABER, P. H. MILLAR, JR., AND H. HARDIN**

Recent studies[1-3] have demonstrated that death in experimental hemorrhagic pancreatitis can be completely or largely prevented by certain antibiotics. This finding indicates that infection is the primary cause of death in dogs afflicted with hemorrhagic pancreatitis produced by the injection of bile into the pancreatic duct. In planning an investigation to evaluate an antitryptic substance in this condition, it became evident that little or no effort had been made in previous work on this problem[4-6] to assess objectively the element of infection, although a comparison of the mortality rate was employed as the gauge of the efficacy of the agent being tested It was felt essential that some manner of producing a non-infected yet lethal hemorrhagic pancreatitis should be devised before embarking on any attempt to clarify the issue relative to the merits of an antitryptic material in pancreatitis. This communication is a presentation of such a method.

METHODS

The experiments were divided into three parts:

Group I. Control pancreatitis using the bile injection technique.

Group II. Preoperative Terramycin and neomycin and pancreatitis using the bile injection technique.

Group III. Preoperative Terramycin and neomycin and pancreatitis using injection of trypsin (Tryptar, Enzar).

The initial two phases consisted essentially of a repetition of the work reported by other investigators with the exception that a combination of Terramycin and neomycin† was selected as the antibiotics. The final stage dealt with our attempts to produce a sterile lethal pancreatitis by the injection of trypsin in dogs protected with the antibiotics.

Th[illegible] treatment during the duration [illegible] received initially a large dose of castor oil followed by Terramycin and neomycin in daily amounts of 1.5 to 3 grams for three to four days prior to operation, the dosage schedule being varied in accordance with the degree of success being attained in sterilizing the stool.

One to two stool specimens were obtained for bacteriologic study preceding the surgery. Acute hemorrhagic pancreatitis was created by a previously described technique,[1,2] illustrated in the drawing, in which bile or trypsin is injected into the accessory pancreatic duct. The volume of bile injected varied from 6 to 10 cc. and the total unitage of trypsin (Tryptar

* From the Department of Surgery, University of Arkansas School of Medicine. This investigation [illegible]

** [illegible]

Phillip [illegible] n and Miss LaVada

† T[illegible]

of Pfi[illegible] ‡ Dr. H. W. Rudel

in generous amounts. [illegible] Upjohn Company

evidence of return of circulation to those areas of the pancreas into which bile leakage had occurred.

COMMENT

From these observations it is evident that extravasation of bile into the interstitial tissues of the pancreas brings about extreme circulatory stasis and that this is a manifestation of the direct action of bile salts on blood vessels. It would seem reasonable to believe that the prolonged anoxia resulting from this complete cessation of pancreatic blood flow is the major factor in determining the subsequent development of pancreatic coagulation necrosis.

This finding has interesting parallels in other forms of experimental pancreatitis,[5-8] recently described, where the evidence points to the primary importance of vascular factors.

SUMMARY

The interstitial injection of bile produces extensive and prolonged circulatory stasis chiefly by direct action on capillary walls and to a lesser degree by causing spasm of vessels possessing a muscular coat. When bile is forcibly injected into the ductal system of the transilluminated rabbit pancreas this rapidly developing vascular injury is readily seen and virtually complete cessation of pancreatic blood flow ensues. The idea is advanced that the parenchymal necrosis which characterizes this experimental form of hemorrhagic pancreatitis is in large measure the result of such interference with local blood flow.

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The bacteriologic analysis was performed by placing the appropriate samples in thioglycollate solution. The tubes were usually read in 24 to 48 hours, but no tube was discarded as being negative until a minimum period of 72 hours had lapsed. The microorganisms were then identified by culturing on differential media. The aerobic organisms were classified mainly as gram-negative, comprising the *E. coli*, Aerogenes and Proteus group, or gram-positive, consisting of *Streptococcus fecalis* and Staphylococcus. The anaerobes were categorized simply as clostridia, no attempts being made to identify the individual strains beyond this simple classification.

RESULTS

The term "sterile" pancreatitis was applied only to those experiments in which the peritoneal exudate cultured at postmortem was sterile. In such instances the bile and portal blood cultures were likewise proven to be uninfected. It was noted early in the control bile pancreatitis studies that an objective bacteriologic assessment of the pancreas was impractical in the presence of a generalized peritonitis. Moreover, it was assumed that a significant degree of infection within the pancreas would be manifested by contiguous spread to the peritoneal cavity and be reflected in a contaminated peritoneal fluid.

Table 1. Mortality and Incidence of Sterile Pancreatitis

GROUP	NO. OF DOGS	MATERIAL INJECTED INTO DUCT	POSTOPERATIVE TREATMENT	MORTALITY	% OF "STERILE" PANCREATITIS AT AUTOPSY
I	12	6–10 cc. bile	None	8 (67%)	2 (25%)
II	11	7–10 cc bile	None	3 (27%)	1 (33%)
III	21	160,000–200,000 U. Tryptar or Enzar	0.2–0 4 Gm. IM Terramycin, 500–750 cc Normal saline IV	19 (90%)	14 (74%)

Group I. Eight of the 12 animals died after 13 to 96 hours. With two exceptions the peritoneal fluid and pancreas were contaminated, predominantly with clostridial organisms. The coli and gram-positive bacteria were less frequently present.

Group II. Three of the 11 animals protected preoperatively with Terramycin and neomycin succumbed to the hemorrhagic pancreatitis induced by bile. Only one had proven sterile pancreatitis. A second dog, which had been given erroneously an excessive amount of bile, died after some 20 hours during the morning hours and was not autopsied until several hours after its demise. Another animal, which probably could have been salvaged by additional antibiotic support in the postoperative period, died of peritonitis on the fourth day. This dog represented the sole instance in this group in which the stool contained, aside from a heavy growth of coli organisms, clostridia despite coverage by antibiotics. The culture of the bile obtained routinely at operation was uniformly sterile.

Group III. The execution of the experiments in this category deviated from the previous two series in two respects. First, the animals received 500 to 750 cc. of intravenous normal saline in the postoperative period in

or Enzar*) from 160,000 to 200,000 in 10 cc. of vehicle. The material was injected with as great pressure as one could reasonably exert with a 10 cc. Luer-Lok syringe through a No. 18 or No. 19 blunt needle. In an effort to secure a more uniform process throughout the entire pancreas, a slight modification was adopted in which the duct leading from the head was injected separately from that draining the body and tail. This maneuver was feasible in approximately 80 per cent of the cases. The pancreas stained a

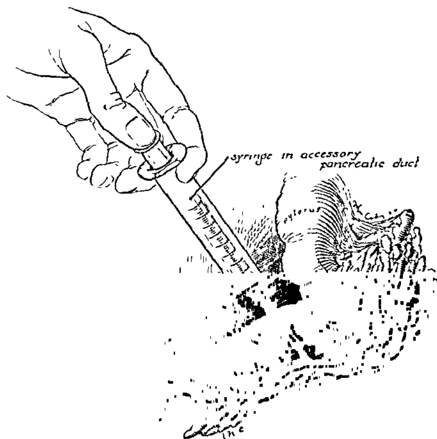


Fig 1. Method of injection of bile or trypsin into accessory pancreatic duct.

mahogany brown following the administration of bile, and an intense purplish-red with trypsin

The animals were closely observed and those succumbing were autopsied as soon as possible. Using sterile technique, cultures of the peritoneal fluid, bile and portal blood were obtained in every instance except in one animal which expired during the early morning hours. The volume of peritoneal exudate was accurately measured in a suction bottle. The process involving the pancreas was graded as to its severity and uniformity, and the specimen sent to Pathology for microscopic study.

During the course of the experiments, routine measurements of the

Bacteriologic Data. The preoperative stools were rendered sterile or almost free of organisms in roughly 50 per cent of the dogs by the antibiotics. Heavy growths of the coliform bacteria as well as aerobic gram-positive organisms were noted in some of the stool specimens. The gas-forming anaerobes persisted in three instances. Initially these failures were attributed to several factors, such as insufficient number of days of treatment, inadequate dosage and hot weather. Sensitivity tests performed in the latter part of this study and in subsequent experiments on the organisms prevalent in the stools revealed a surprisingly high rate of resistance of the colon bacilli and gram-positive strains to Terramycin and, to a lesser extent, of *E. coli* to neomycin. The former drug was effective in eliminating the clostridial group of organisms, a task which neomycin appears to be incapable of accomplishing.

The bacteriology of the peritoneal fluid in the five infected deaths revealed a predominance of the colon group of bacilli except in one case in which clostridia were found. This latter organism had been cultured in the feces of this particular animal. In three of the five animals dying with bacterial peritonitis, the stools were sterile or only slightly contaminated prior to operation Conversely it has been noted on several occasions that a non-infected form of fatal pancreatitis resulted despite the presence of heavy growths of *E. coli* and, in two instances, anaerobes in the stools.

DISCUSSION

It has been known for many years that microorganisms, chiefly clostridia and *E. coli*, reside in the liver, pancreas and other organs of supposedly healthy mongrels. Basing his opinion on experimental evidence in dogs, Dragstedt[7] postulated in 1934 that the toxemia of acute pancreatic necrosis was due to the absorption of toxic substances produced in the necrotic pancreas by the action of bacteria. That this view has not been universally shared is evident by the fact that many investigators have failed to consider infection as an important factor in influencing the mortality in hemorrhagic pancreatitis in dogs Recent investigations have conclusively proven that certain antibiotics are able to prevent or reduce deaths in experimental hemorrhagic pancreatitis. Persky and his co-workers[1] have demonstrated a marked increase in the incidence of pathogenic bacteria, particularly clostridia, in the peritoneal exudate, pancreas, liver and portal blood of dogs dying from bile-induced pancreatitis; moreover, they obtained a 100 per cent survival rate in their animals, as compared to a control fatality rate of 90 per cent, by oral Aureomycin administered either before or after the production of the pancreatitis. Penicillin,[2] intravenous Aureomycin[1] and neomycin[3] were found to be less effective It was concluded from these data that the bacteria inhabiting the intestines were largely responsible for the virulent infection leading to a lethal outcome by invading other areas of the body following the onset of bile pancreatitis The results of our studies are essentially in agreement with these conclusions.

In an effort to create a fatal yet sterile form of pancreatitis, trypsin was selected as the inciting agent. Of immediate concern at the outset was the possible effect on the dogs of the sudden absorption of the 160,000 to 200,-000 U. of trypsin being administered through the pancreatic duct. It has been found that a comparable dose—13,000 U. per kilogram—can be given intravenously to dogs in one minute without any lasting deleterious se-

keeping with the plan of employing a like volume of saline as the vehicle for the antitryptic agent to be subsequently evaluated. Secondly, the last 13 dogs received an additional 0.2 to 0.4 grams of intramuscular Terramycin* *after surgery in an attempt to attain more consistently the previously stated* objective of eliminating infection as a factor in influencing the mortality in these experiments.

Only two of the 21 dogs injected with trypsin survived the severe pancreatic inflammation, there being 16 consecutive fatalities prior to the first survivor. Of the 19 deaths, 14 fulfilled the bacteriologic criteria of a "sterile" lethal pancreatitis. The peritoneal fluid of the remaining five animals was found to be infected.

The average survival time in the 19 deaths was approximately ten hours,



FIG. 2 Gross specimen of trypsin pancreatitis The head of the pancreas is not as severely involved Areas of gross necrosis are visible.

excluding one animal which lived for 4½ days. The next longest survivor lasted 22 hours. The clinical picture in the dogs appeared to be that of a progressive shock state, characterized by a rapid, weakening pulse, tachypnea, increasing lethargy and exhaustion terminating at times in a stuporous condition, although some of the animals remained alert and awake until shortly before they expired Two of the animals never recovered consciousness and died rapidly within a few hours. The postmortem findings were striking There was a large amount, varying from 500 to 900 cc, of grossly bloody fluid, a marked contrast to the thinner, amber-colored fluid present in the bile-induced pancreatitis. The pancreas was discolored a dark purplish red and swollen, with areas of gross necrosis. Fat necrosis was not a conspicuous feature The evidence uncovered at autopsy seemed adequate to explain the picture of clinical shock observed in the animals.

* The intramuscular Terramycin was donated by Pfizer Laboratories.

STUDIES OF PITUITARY FACTORS AFFECTING THE PANCREATIC INSULAR TISSUE*

KANWAL K. KAPUR, STANLEY C. SKORYNA, AND DONALD R. WEBSTER**

Considerable knowledge has accumulated with reference to the factors affecting pancreatic insular tissue. Anselmino et al.[1] were the first to establish the existence of an insulotrophic factor in the anterior pituitary. Availability of purified growth hormone and perfection in the technique of hypophysectomy have created new possibilities in the evaluation of these factors. The status of the insular tissue in the endocrine cycle is still controversial. In this experiment an attempt was made to evaluate the hypophyseal control of the islets of Langerhans.

MATERIALS AND METHODS

Sixty-five male rats weighing between 180 and 220 grams of the Royal Victoria Hospital hooded strain were used. The animals were maintained on a synthetic purina meal diet. The colony was divided into three groups.

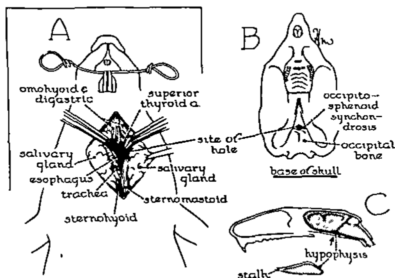


Fig. 1. Diagrammatic illustration of technique of hypophysectomy.

Group I. This control group consisted of 25 animals. Five animals were sacrificed at biweekly intervals starting from the first week.

Group II. Consisted of 20 animals, growth hormone was administered to these animals at the dosage of 4 mg per 100 gm. body weight daily. Five animals were sacrificed at intervals corresponding to the controls.

* From the Department of Experimental Surgery, McGill University, Montreal, Quebec, Canada. This work was supported by a grant-in-aid from the National Cancer Institute of Canada

** The authors wish to thank Dr G. C. McMillan for his help in the interpretation of the histologic material and Dr. C. W. MacMillan for the statistical analysis.

quelae, although there is a transient fall in blood pressure[8] An increase of the dosage to 25,000 U. per kilogram has been followed by the death of the animal in shock within 15 to 45 minutes, presumably from peptone shock.[8] However, it has been found that massive amounts of trypsin given intravenously up to 100,000 U. per kilogram to dogs did not cause shock provided that the rate of administration was limited to 30 to 40 drops per minute of a solution containing 1000 U of trypsin per cc.[9] Our experiences in administering 200,000 U of Enzar (intravenous crystalline trypsin) to dogs likewise indicated that this amount of trypsin was not obviously toxic if introduced over a period of several minutes. The clinical course of the animals, the response of the antitryptic activity, and the autopsy findings previously described all tended to corroborate the impression that death in these animals was attributable to the effects of the hemorrhagic pancreatitis rather than acute trypsin toxicity. There appeared to be no appreciable differences detected in the clinical and pathologic pictures produced by the two forms of trypsin, Tryptar and Enzar.

In the light of the knowledge pertaining to the opposing roles of bacterial infection on one hand and the antibiotics on the other in bile-induced pancreatitis in dogs, it becomes apparent that the data relating to certain phases of the pancreatitis problem, especially those pertaining to therapy, may not be entirely valid in those experiments which fail to consider objectively the harmful action of bacteria. Certainly future studies along these lines must necessarily provide for rigid control and assessment of this factor.

SUMMARY

1. Bacteriologic studies indicate that pathogenic bacteria are present in the peritoneal exudate, bile and other tissues of dogs succumbing to hemorrhagic pancreatitis, produced by the standard bile injection method.

2. Oral Terramycin and neomycin are effective in reducing significantly the mortality rate in bile-induced pancreatitis.

3. A method of creating a sterile, lethal form of hemorrhagic pancreatitis is presented.

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increase in body weight was observed in this group. The scatter curve did not show significant change in the range of islet size distribution.

Group II. Considerable variation in the size and shape of islets was observed, due to the appearance of a large number of new islets and formation of giant islets. The beta cells showed a deeper staining with prominence of cytoplasmic granules. The observed increase in the number of smallest islets and larger islets was found to be statistically significant ($t = <0.01$) after 5 weeks of growth hormone administration. The increase in body weight was almost twice greater than in the control group. The blood sugar determinations showed no significant changes.

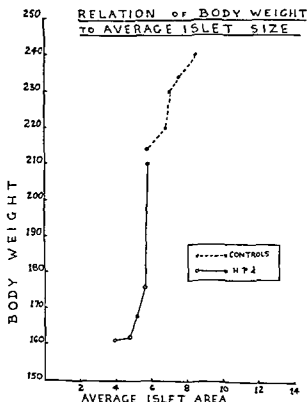


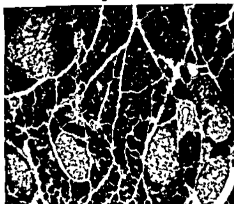
Fig. 5. Curve showing relation of body weight to average islet size in control and hypophysectomized animals.

Group III. In this group a gradual decrease in the average islet size was observed and found to be statistically significant ($t = <0.01$) in the fifth week following hypophysectomy. The range of the islet size distribution also showed a significant decrease. There occurred a progressive atrophy of the pancreatic tissue. Both the exocrine and endocrine portions underwent a reduction in size. The islets appeared to lie closer to one another in the acinar tissue. The larger sized islets became scarce and the smaller islets showed a reduction in size. The cytoplasm of the granular cells appeared more homogeneous than in normal animals. A 15 to 20 per cent drop in body weight occurred after the first week of the experiment, followed by a gradual

Group III. Consisted of 20 animals which were hypophysectomized under light ether anesthesia by a parapharyngeal approach using modified Smith technique[2] Blood sugar determinations were carried out by Folin's micro method.[2]

Histologic sections were prepared with hematoxylin-eosin stain and the modified Gomori's chromium hematoxylin stain.[2] The islets were measured

Fig. 2.



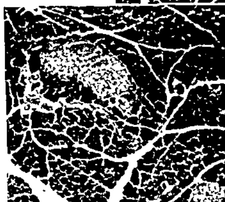


Fig 3

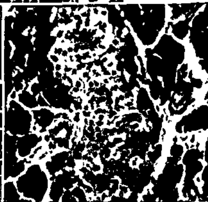


Fig. 4.

Fig. 2. Photomicrograph of rat pancreas 5 weeks following hypophysectomy, showing reduction in the size of exocrine and endocrine portions The islets appear to lie closer to one another

Fig 3. Photomicrograph of rat pancreas 7 weeks after growth hormone administration, showing an increased range in islet size variation

Fig 4. Same as Figure 3 (under a magnification of 250), showing an islet containing compactly arranged deeply staining beta cells.

by a micrometric method.[2] One hundred islets were measured in each animal and the average size of the islet calculated in each group. The hundred islets measured in each animal were separated into different size groups and scatter curves drawn to compare the range of islet size distribution in all the three groups

RESULTS

Group I (Controls). A gradual increase in average islet size paralleling

absence of large islets encountered in the controls at the start of the expe ment. The evidence of islet tissue atrophy based on altered granulati alone could be contradicted by the decreased food intake in the hypoph sectomized animals, because similar changes in the beta cells during starv tion have been reported by Tegning.[6] The decrease in food intake does n appear to be significant when the associated decrease in body weight taken into consideration. Results of this experiment are in agreement wi the findings of Salter and Best,[7] who were able to restore growth in hyp

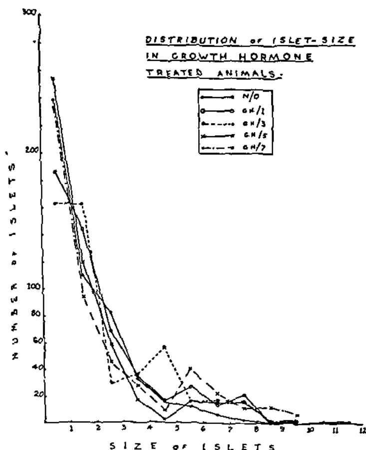


Fig. 7. Curves showing distribution of islet size in growth hormone treated animal

physectomized animals on insulin administration. It can be postulated t hypophysectomy, by reducing the amount of available insulin, *results* diminished food intake leading to decrease in body weight.

Growth hormone treated animals showed an apparent *increase in the i* tissue content, as evident from the appearance of a large number of n small islets and a greater number of larger islets. The beta cells showe deeper staining, with structural changes indicating increased proliferati Similar findings in rats have been reported by a number of workers.[8-11] T diabetogenic action of growth hormone could be interpreted as a result an excessive hormonal stimulation of a process which normally results

decrease averaging 5 per cent in the later weeks. Blood sugar showed an average fall of 10 mg. in the first week following hypophysectomy. This lowered level remained almost constant in the later weeks of the experiment

DISCUSSION

Krichesky[3] reported an increase in the amount of islet tissue in rats following hypophysectomy. Similar findings were achieved in newts by Adams and Ward.[4] According to Haist,[5] no islet tissue atrophy occurred after hypophysectomy, though this author reported inhibition of islet growth associated with inhibition of body weight.

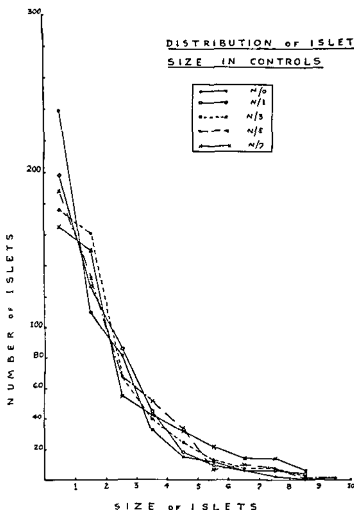


Fig 6 Curves showing distribution of islet size in controls.

Our findings are in agreement with those of Haist regarding the inhibition of islet growth In addition, there is definite evidence of islet tissue atrophy, as is apparent from the reduction in the size of the islet cells, their more homogeneous cytoplasm, a decrease in the average islet size and complete

in the anterior pituitary and the possible role of such a factor in carcinogenesis have been discussed.

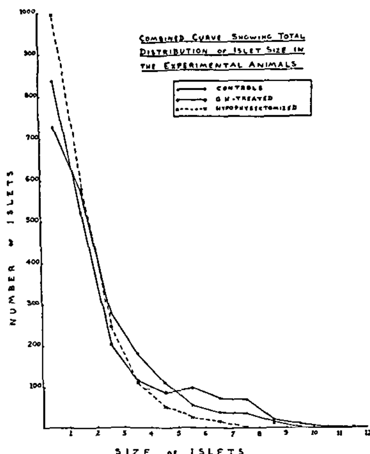


Fig. 9. Combined curve showing comparative distribution of islet size in experimental animals.

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increased growth rate. The normal rat appears to have an adequate islet tissue reserve to supply additional insulin in order to maintain a normal blood sugar level. Thus the predominant action of growth hormone in such an animal is insulotrophic The inhibition of tumor growth following 2-AAF administration in hypophysectomized rats observed in our laboratories[12]

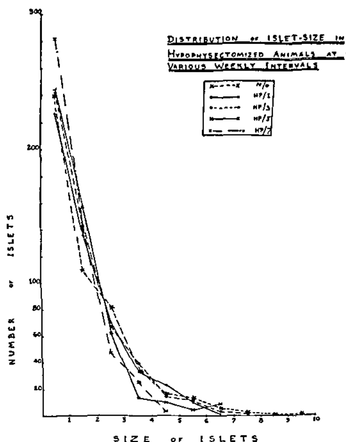


Fig 8 Curves showing distribution of islet size in hypophysectomized animals.

may be partially due to lack of an insulotrophic factor which results in inhibition of growth.

SUMMARY AND CONCLUSIONS

to follow hypophysectomy.

3. An increase in the average islet size and the range of distribution was observed to follow growth hormone administration, as measured by a micrometric method.

4. Experimental results suggesting the presence of an insulotrophic factor

Simons[5] in 1915 and by Patric, Pyle and Vale[6] in 1917 by simpler means but with short-term evaluations.

Tripodi and Sherwin[7] (1934) and Person and Glenn[8] (1939) performed pancreatogastrostomy in dogs to determine proper disposition for the pancreas remaining after radical pancreaticoduodenectomy for malignant disease. In 1944 Ferguson and Wangensteen[9] did extensive studies to determine the type of pancreatic duct anastomosis which would provide the best results, and concluded that direct suture over a temporary tube was most satisfactory.

The utilization of retrograde drainage from the tail of the pancreas was first successfully employed in a human case by Link[10] in 1911, when he created a fistula between the pancreatic tail and the abdominal wall in a patient with pancreatolithiasis.

METHOD

To help determine the efficiency of pancreatic drainage by retrograde pancreatojejunostomy and the most suitable technique to be employed, 24 dogs divided into four groups were studied. A comparison of implantation of the tail of the pancreas into a loop of jejunum, with implantation into a Roux-Y construction was planned. The need for a direct suture anastomosis of pancreatic duct to jejunal mucosa was also evaluated. Finally, the fate of the anastomosis after release of the obstructing element at the head of the gland was determined.

Group 1. The pancreatic ducts (main and accessory) were identified by transduodenal cannulization and were doubly ligated with silk and divided. The pancreatic head was well separated from the duodenum and the omentum was interposed to help prevent recanalization of the ducts into the duodenum. The pancreatic tail was excised and the open end of the pancreas was implanted into a loop of jejunum The pancreas was anchored to the jejunum with a single row of 4-0 silk sutures.

Group 2. The procedure was the same as used with group 1 except that the open end of the pancreas was implanted into the arm of a Roux-Y construction of jejunum. This defunctionalized arm was made 18 to 20 cm. in length.

Group 3. The main and accessory ducts were ligated as before. Five to seven days were allowed to elapse to permit dilatation of the pancreatic duct. At the second stage procedure, the pancreatic tail was excised and the dilated pancreatic duct was anastomosed directly to jejunal mucosa with interrupted 6-0 silk sutures. No more than 4 or 5 sutures were required for the anastomosis. The glandular portion next to the pancreatic duct was then buttressed to the serosal surface of the jejunum.

Group 4. *First Stage.* The pancreatic ducts were temporarily occluded by loops of fine polyethylene tube passed around each duct and pulled tightly against the anterior abdominal wall.

Second Stage. After 5 to 7 days the pancreatic tail was excised and a direct anastomosis of pancreatic duct to jejunal mucosa was done as in group 3.

Third Stage. After 2 to 7 days the polyethylene ties occluding the main and accessory pancreatic ducts were released.

Serum amylase levels were determined in all animals pre- and postopera-

AN EXPERIMENTAL STUDY OF RETROGRADE PANCREATOJEJUNOSTOMY*

JOSEPH A. BONTA

One of the most perplexing problems to the surgeon is the patient with chronic pancreatitis who has run the gamut of surgical attacks including partial gastric resection, vagotomy, diversion of biliary flow, sphincterotomy, etc, but who continues to have debilitating pain from unrelenting progression of his disease. In general, the basis for the majority of these approaches has been an attempt at diminution of pancreatic stimulation, prevention of biliary reflux into the pancreas, or promotion of better pancreatic drainage. Although based on sound physiologic principles, they have too often failed to provide adequate or lasting relief.

Although considerable controversy exists concerning the pathogenesis of chronic pancreatitis, it is reasonable to assume that any obstruction to the outflow of pancreatic secretion would seem to perpetuate the disease. (Ligation of the pancreatic ducts and forced feeding will produce pancreatitis)[1] The dilated duct so often found at surgery and the study of autopsy specimens[2] in patients with chronic pancreatitis lends further support to the supposition that an obstructive element is present and may represent another factor capable of inciting repeated bouts of pancreatitis, particularly in the patient with pseudocyst formation or pancreatic calcification

Two patients recently presented themselves to this clinic with far advanced chronic pancreatitis, exhibiting calcifications and pseudocyst formation (reported elsewhere).[3] Each had had multiple operative procedures, as detailed above, with only temporary benefit. In order to forestall further progression of the disease, an attempt to establish free pancreatic drainage was made by resection of the tail of the pancreas and direct anastomosis of the pancreatic duct to the jejunum, thus providing an unobstructed route for pancreatic secretions In one patient the anastomosis was accomplished with a Roux-Y construction of jejunum and in the other with a simple loop of jejunum. Each patient has been free of acute pain for approximately one year and has been gainfully employed. Diodrast injection into a catheter previously placed in the pseudocyst demonstrated travel of the dye into the bowel by both routes, i e., the normal route into the duodenum and [illegible] hallenging [illegible] duration [illegible] ciple was questioned, which introduced the problem of determining the most satisfactory means of establishing the retrograde pancreatojejunostomy if it proved useful

The feasibility of such an anastomosis was suggested by animal experimentation in 1909 by Coffee,[4] whose technique required the construction of a double lumen of jejunum fashioned into a single receptacle after the technique of a Finney pyloroplasty. Further study was done by Sweet and

* From the Department of Surgery and the Surgical Laboratories, Ohio State University College of Medicine, Columbus. This study was aided in part by a grant from the National Institute of Health, Bethesda, Maryland

animal died from leak at the site of anastomosis with peritonitis and was also discarded from the study.

Group 3 (Six Animals). After sacrifice at 2 weeks, 3 weeks, 3 months, 5 months, 5½ months, and 6 months, all anastomoses were patent and the pancreas in each was soft and pliable. Microscopically, there was no appreciable fibrosis except at the head of the gland where the ducts had

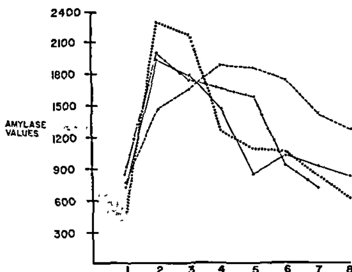


Fig 2. The amylase response in all groups.

Table 1. Compiled Results of All Groups

	DOG NO	ANASTOMOSIS	PANCREATIC FIBROSIS	DURATION
Group 1	1	Patent	None	5 weeks
	2	Patent	None	6 weeks
	3*	Closed	Moderate	17 weeks
	4	Closed	Moderate	30 weeks
	5	Closed	Moderate	30 weeks
	6	Patent	Slight	34 weeks
Group 2	7	Patent	None	2 weeks
	8	Patent	None	4 weeks
	9*	Closed	Slight	8 weeks
	10	Patent	None	18 weeks
	11	Closed	Moderate	26 weeks
	12	Closed	Moderate	26 weeks
Group 3	13	Patent	None	2 weeks
	14	Patent	None	3 weeks
	15	Patent	None	3 months
	16	Patent	Slight	5 months
	17	Patent	Slight	5 months
	18	Patent	None	6 months
Group 4	19	Closed	None	3 months
	20	Closed	Moderate	3 months
	21	Closed	Slight	4 months
	22	Closed	Slight	4 months
	23	Closed	None	4 months
	24	Closed	Moderate	4 months

* Recanalization of pancreatic duct into duodenum was demonstrated.

tively in order that they might reflect the degree of pancreatitis incited, if present, and its response to the staged procedure. Pancreatic biopsies were obtained at sacrifice and at intermediate stage procedures to demonstrate the degree of fibrosis as an index of the adequacy of pancreatic drainage.

Animals were sacrificed at varying intervals from two weeks to seven months. The operative specimens (duodenum, pancreas and jejunum) were removed en bloc, and the pancreatic duct was cannulated with fine polyethylene tubing for injection of dye to determine the patency of the anastomosis. Gross dissection and serial section of the anastomosis was done to confirm the dye injection method.

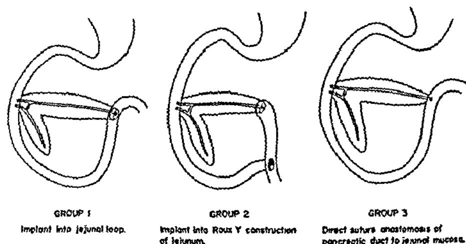


Fig. 1. Diagrammatic representation of the technical variations of Groups 1, 2, and 3

RESULTS

Group 1 (Six Animals). These dogs were sacrificed at 5 weeks, 4 months, 4½ months, 7 months, 7½ months and 8 months. The implant had closed in three dogs. In one (No. 3) there was recanalization of the pancreatic ducts into the duodenum. The pancreas was soft and pliable in all except two cases in which no outlet for pancreatic secretions could be demonstrated. In these, pancreatic fibrosis was moderately heavy but an appreciable quantity of normal-appearing glandular tissue remained on microscopic examination.

Group 2 (Six Animals). These animals were sacrificed at 2 weeks, 4 weeks, 8 weeks, 4½ months, 6 months and 6½ months. Again there was one animal (No. 9) in which the implant had failed and recanalization of pancreatic drainage into the duodenum was demonstrated. There were also two animals in which no outlet for pancreatic secretion could be found and in these cases the pancreas was indurated and fibrotic. In the remaining three animals the implant functioned well and the pancreas in each was soft and pliable. The portion of pancreas which had been implanted into the lumen of the bowel had been digested and the duct lay flush with the bowel mucosa. One animal died on the fifth postoperative day of extensive pancreatitis and was not useful for evaluation of the anastomosis. Another

animal died from leak at the site of anastomosis with peritonitis and was also discarded from the study.

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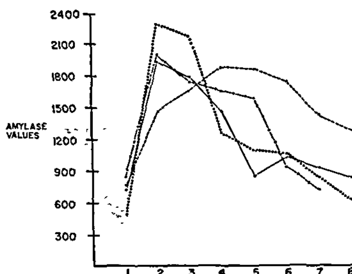


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	14	Patent	None	3 weeks
	15	Patent	None	3 months
	16	Patent	Slight	5 months
	17	Patent	Slight	5 months
	18	Patent	None	6 months
Group 4	19	Closed	None	3 months
	20	Closed	Moderate	3 months
	21	Closed	Slight	4 months
	22	Closed	Slight	4 months
	23	Closed	None	4 months
	24	Closed	Moderate	4 months

* Recanalization of pancreatic duct into duodenum was demonstrated.

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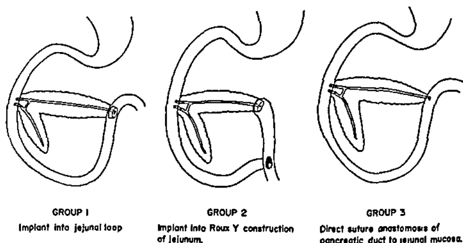


Fig. 1 Diagrammatic representation of the technical variations of Groups 1, 2, and 3

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Group 1 (Six Animals). These dogs were sacrificed at 5 weeks, 4 months, 4½ months, 7 months, 7½ months and 8 months. The implant had closed in three dogs. In one (No 3) there was recanalization of the pancreatic ducts into the duodenum. The pancreas [illegible]

[illegible] but an appreciable quantity of normal-appearing glandular tissue remained on microscopic examination.

Group 2 (Six Animals). These animals were sacrificed at 2 weeks, 4 weeks, 8 weeks, 4½ months, 6 months and 6½ months. Again there was one animal (No 9) in which the implant had failed and recanalization of pancreatic drainage into the duodenum was demonstrated. There were also two animals in which no outlet for pancreatic secretion could be found and in these cases the pancreas was indurated and fibrotic. In the remaining three animals the implant functioned well and the pancreas in each was soft and pliable. The portion of pancreas which had been implanted into the lumen of the bowel had been digested and the duct lay flush with the bowel mucosa. One animal died on the fifth postoperative day of extensive pancreatitis and was not useful for evaluation of the anastomosis. Another

interesting that the Roux-Y did nothing to prevent failure of the implant and was a superfluous maneuver in these animals. Since the anastomosis failed in all animals of group 4 (only temporary occlusion of pancreatic ducts), one might predict failure for retrograde pancreatojejunostomy in its clinical application, since obstruction to pancreatic flow into the duodenum in most patients with far advanced chronic pancreatitis is incomplete. Also, any obstruction due to edema would probably subside following such an operation. However, the fibrosis, calcification and cyst formation probably represent the major elements of obstruction and are relatively irreversible. Final

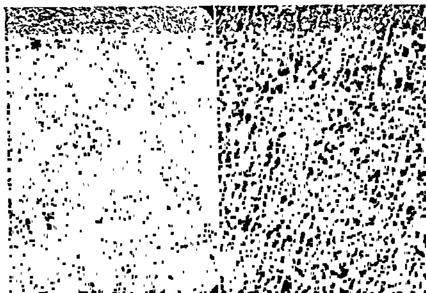


Fig. 4 Photomicrograph of pancreatic biopsy of (*a*) dog No 5, showing appreciable fibrosis after failure of the pancreatojejunostomy, (*b*) dog No. 18, showing no fibrosis after 6 months with functioning anastomosis

evaluation can be made only after further clinical application and long-term study.

CONCLUSIONS

1. Retrograde pancreatojejunostomy will provide adequate outlet for pancreatic secretions.
2. Direct suture anastomosis of pancreatic duct to jejunal mucosa is superior to implantation of the gland into the lumen of the bowel.
3. There are no apparent advantages to the utilization of the Roux-Y principle in these animals.
4. The pancreatojejunostomy will close if the obstruction at the head of the pancreas is totally relieved.

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been ligated (as was present in all groups). At the pancreatojejunostomy there was a firm fibrous union joining the tail of the gland to the wall of the jejunum. One additional animal eviscerated on the sixth postoperative day and was eliminated from the study.

Group 4 (Six Animals). A striking similarity in the results of all six animals of this group was the failure of the anastomosis. Since the pancreatic ducts at the duodenum were only temporarily occluded, this group confirmed the supposition that the by-pass route would close if there were no need for it.

The amylase response was grossly similar in all groups (Fig. 2) in that an initial rise to 2 to 4 times normal was evident on the first postoperative day and required 5 to 7 days for a gradual return to normal range. This is interpreted as a response to ligation of the main and accessory ducts

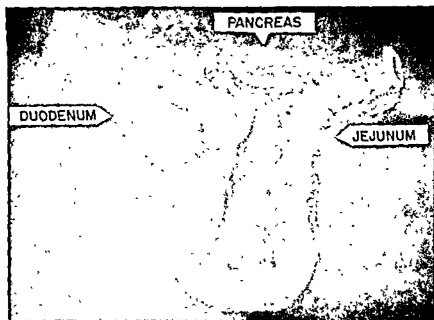


Fig 3 X-ray representation of retrograde flow of dye through the pancreatojejunostomy in dog No. 18.

which necessitated dissection of pancreatic glandular tissue, with a local postoperative or "traumatic" pancreatitis. The fibrosis in this area after sacrifice further suggested such a response. Study of the individual amylase curves gave no clue to the maintenance or failure of the anastomosis Table 1 depicts the average response in each of the four groups.

COMMENT

Although short-term examination (up to 6 weeks) of the "implant" technique (groups 1 and 2) would imply a satisfactory result, the final evaluation is less striking (50 per cent failure in each group). One might assume that progressive cicatrization which eventually closed the anastomosis in half the cases in groups 1 and 2 was not allowed to take place when a direct duct to jejunal mucosa anastomosis was used, as in group 3. It is

the dog.[10] Its action on human pancreatic secretion has not yet been demonstrated.

METHOD

The patients included in this investigation were drawn from the Private and Ward Services of the Mount Sinai Hospital, New York City. Patients without evidence of pancreatic disease (14) as well as those with proved pancreatic inflammations (4) were studied. The procedure was the same for both groups.

All patients, 18 in number, were tested in the fasting state. A double-lumened gastroduodenal tube was positioned fluoroscopically at the ligament of Treitz. Gastric and duodenal drainage were obtained by continuous suction with a Gumco pump. A secretin test was done using the standard dosage of 1.0 clinical units per kilogram of secretin (Lilly) and a collection period of 120 minutes.[11] Then, the sodium salt of Diamox (Lederle) was administered intravenously in dosage ranging from 10 to 121 mg. per kilogram. The Diamox was dissolved in a liter of distilled water and the infusion given over a period of approximately one hour. Following this, a second secretin test was done, completing the double-secretin test technique.[12]

RESULTS

Having no previous knowledge of the effect of the drug on human pancreatic secretion and hence no indication of the optimum dose range, it was expedient for us to administer Diamox to successive patients in stepwise fashion starting with 500 mg and progressing to 9000 mg. In this series there were no side reactions other than marked diuresis in all patients and mild headache in three patients. No alterations in pulse, blood pressure, or respiration were noted in any patient No changes in the *p*H of the blood, its CO_2 capacity, or the blood electrolyte (Na, K, Cl) concentrations were observed under the experimental conditions.[13]

Volume Response. Table 1 (columns 1 to 3) shows the alteration in volume response effected by Diamox It will be noted that there is a progressive diminution of volume flow after secretin with increasing dosage of the carbonic anhydrase inhibitor. In the last seven patients, who received 80 or more mg. per kilogram, the percentage decrease in volume flow obtained in the secretin tests averaged well over 75 per cent. Actually this does not indicate that Diamox administration resulted in a 75 per cent blockage of the pancreatic volume response to secretin. One must consider that the unstimulated basal rate of volume secretion is about 1 ml. per minute. Thus, these patients should have secreted at least 80 ml. during the collection periods of the secretin test even at basal rates of secretion. The data reveal that every patient to whom 50 or more mg. per kilogram was given had a post-Diamox volume secretion less than 80 ml., many exhibiting extremely low volumes such as 18 ml, 22 ml., 33 ml., etc. These findings suggest that not only does Diamox, at high dosage levels, block the volume pancreatic response to secretin but it also appears to depress the basal rate of fluid secretion. The volume effect obtained in patients without pancreatic disease was the same as that observed in patients with pancreatitis.

Bicarbonate Secretion. Table 1 (columns 4 to 6) illustrates the effect of intravenous Diamox on the bicarbonate secretion of the pancreas. It is clear

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INHIBITION OF HUMAN PANCREATIC SECRETION BY DIAMOX (CARBONIC ANHYDRASE INHIBITOR)*†

Therapeutic Implications in Pancreatitis

DAVID A. DREILING AND HENRY D. JANOWITZ‡

The local pathology of acute pancreatitis results from the digestive action of the pancreatic ferments which have escaped from the ductal system Medical therapy, in the past, has been directed towards diminishing the secretion of these ferments and towards their inactivation. This is the physiologic basis for the use of enzyme inhibitors (soybean trypsin inhibitor),[1] vagolytic drugs (banthine),[2] and for various neurologic procedures (epidural block,[3] sympathetic block,[4] etc.).

The emphasis on pancreatic ferments is easily understood: it derives from the classic theory of pathogenesis, the common channel theory of Opie,[5] which stresses the activation of pancreatic ferments within the pancreatic ducts by refluxed bile and then the escape of these activated ferments from the ductal system. In recent years, more attention has been paid to the mechanism whereby the ferments are able to escape from the ducts.[6] The most cogent explanation offered for this process is the theory in which enzyme escape is accounted due to the hydrostatic pressure engendered by hypersecretion of the pancreas against ductal obstruction.[6,9] If the dynamics of this hypothesis are correct, the fluid secretion of the pancreas assumes great importance in the etiology of acute pancreatitis and the ability to inhibit fluid secretion would naturally have great significance in the therapy of this disorder.

The present investigation is a study of the effect of Diamox (2-acetylamino-1,3,4-thiadiazole-5-sulfonamide) upon human pancreatic secretion. This drug, a potent carbonic anhydrase inhibitor, has been previously shown to diminish the pancreatic volume and bicarbonate responses to secretin in

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† The Diamox used was supplied by Dr. James D Gallagher, Lederle Laboratories The Secretin used was donated by Dr. C. G. Weigand, the Eli Lilly Company.
‡ With the technical assistance of Miss Alice Klein, B.S.

the dog.[10] Its action on human pancreatic secretion has not yet been demonstrated.

METHOD

The patients included in this investigation were drawn from the Private and Ward Services of the Mount Sinai Hospital, New York City. Patients without evidence of pancreatic disease (14) as well as those with proved pancreatic inflammations (4) were studied. The procedure was the same for both groups.

All patients, 18 in number, were tested in the fasting state. A double-lumened gastroduodenal tube was positioned fluoroscopically at the ligament of Treitz. Gastric and duodenal drainage were obtained by continuous suction with a Gumco pump. A secretin test was done using the standard dosage of 1.0 clinical units per kilogram of secretin (Lilly) and a collection period of 120 minutes.[11] Then, the sodium salt of Diamox (Lederle) was administered intravenously in dosage ranging from 10 to 121 mg. per kilogram. The Diamox was dissolved in a liter of distilled water and the infusion given over a period of approximately one hour. Following this, a second secretin test was done, completing the double-secretin test technique.[12]

RESULTS

Having no previous knowledge of the effect of the drug on human pancreatic secretion and hence no indication of the optimum dose range, it was expedient for us to administer Diamox to successive patients in stepwise fashion starting with 500 mg. and progressing to 9000 mg. In this series there were no side reactions other than marked diuresis in all patients and mild headache in three patients. No alterations in pulse, blood pressure, or respiration were noted in any patient. No changes in the pH of the blood, its CO_2 capacity, or the blood electrolyte (Na, K, Cl) concentrations were observed under the experimental conditions.[13]

Volume Response. Table 1 (columns 1 to 3) shows the alteration in volume response effected by Diamox. It will be noted that there is a progressive diminution of volume flow after secretin with increasing dosage of the carbonic anhydrase inhibitor. In the last seven patients, who received 80 or more mg. per kilogram, the percentage decrease in volume flow obtained in the secretin tests averaged well over 75 per cent. Actually this does not indicate that Diamox administration resulted in a 75 per cent blockage of the pancreatic volume response to secretin. One must consider that the unstimulated basal rate of volume secretion is about 1 ml. per minute. Thus, these patients should have secreted at least 80 ml. during the collection periods of the secretin test even at basal rates of secretion. The data reveal that every patient to whom 50 or more mg. per kilogram was given had a post-Diamox volume secretion less than 80 ml., many exhibiting extremely low volumes such as 18 ml., 22 ml., 33 ml., etc. These findings suggest that not only does Diamox, at high dosage levels, block the volume pancreatic response to secretin but it also appears to depress the basal rate of fluid secretion. The volume effect obtained in patients without pancreatic disease was the same as that observed in patients with pancreatitis.

Bicarbonate Secretion. Table 1 (columns 4 to 6) illustrates the effect of intravenous Diamox on the bicarbonate secretion of the pancreas. It is clear

Table 1. Total Volume Secretion, Total Bicarbonate Secretion, Maximum Bicarbonate Concentration, and Total Amylase Secretion Following Secretin before and after the Administration of Diamox Intravenously to Patients with and without Pancreatic Disease

	DIAGNOSIS	DOSAGE mg /kg	TOTAL VOLUME SECRETION Pre-Diamox ml / 80 min	Post-Diamox ml / 80 min	% Change	TOTAL BICARBONATE SECRETION Pre-Diamox mEq / 80 min	Post-Diamox mEq / 80 min	% Change	MAXIMUM HCO_3 CONCENTRATION Pre-Diamox mEq / L	Post-Diamox mEq / L	% Change	TOTAL AMYLASE SECRETION Pre-Diamox u /kg	Post-Diamox u /kg	% Change
1	Duodenal ulcer	10	182	143	−21	18.8	15.6	−17	122	130	+ 7	11.8	14.4	+22
2	Post-chol syndrome	10	136	131	− 4	10.1	11.1	+10	91	104	−14	23.0	19.6	−15
3	Psychoneurosis	14	221	160	−28	20.6	14.4	−30	122	118	− 3	24.0	18.3	−22
4	Post-chol syndrome	17	128	111	−13	10.0	6.0	−40	118	98	−17	18.7	12.2	−35
5	Migratory phlebitis	30	153	96	−38	10.6	6.4	−40	90	83	− 8	10.3	11.3	+10
6	Abdominal pain, etiology?	31	219	127	−42	13.5	4.8	−64	94	62	−34	18.0	13.2	−27
7	Ulcerative colitis	50	287	101	−61	21.6	6.6	−69	113	82	−27	20.3	10.3	−50
8	Subacute pancreatitis	53	85	49	−41	4.5	2.5	−44	71	88	+10	9.0	8.5	− 6
9	Amebiasis	56	126	33	−74	9.9	2.5	−74	109	95	−13	20.8	6.8	−67
10	Chronic pancreatitis	63	118	67	−43	5.8	2.7	−53	56	42	−25	0.6	0.3	−50
11	Chronic cholecystitis	81	161	22	−86	7.8	1.3	−83	98	86	−12	13.3	2.7	−80
12	Chronic pancreatitis	85	71	18	−75	4.9	0.6	−88	71	42	−39	10.9	2.9	−73
13.	Post-chol syndrome	91	134	37	−72	10.1	1.1	−89	100	35	−65	11.0	3.9	−65
14	Chronic pancreatitis	103	150	28	−81	7.5	1.6	−79	76	75	− 1	22.3	12.5	−44
15	Duodenal ulcer	105	183	40	−80	12.4	2.6	−79	127	90	−29	6.3	4.3	−32
16	Psychoneurosis	108	137	75	−63	12.1	3.6	−70	120	110	− 9	13.4	11.9	−11
17	Duodenal ulcer	112	209	48	−77	16.4	3.1	−81	120	94	−22	11.2	7.8	−30
18.	Psychoneurosis	121	184	48	−73	11.2	2.7	−76	94	95	+ 1	13.8	10.2	−23

Underlining denotes patients with pancreatic disease
Post-chol = Post-cholecystectomy

that the rate of bicarbonate secretion following secretin is greatly diminished by Diamox. In the dosage ranges above 80 mg. per kilogram, the decrease in total bicarbonate secretion for the test periods may be above 70 per cent. Now, in the resting state, the pancreas secretes about 4 0 mEq. in 80 minutes. Seven of the patients without pancreatic disease, all having received more than 50 mg. per kilogram, have secreted less than this basal value even following secretin. The conclusion can be drawn that Diamox not only blocks the bicarbonate response of the pancreas to secretin but is capable of suppressing the basal rate of bicarbonate secretion.

Bicarbonate Concentration. Diminution of total bicarbonate production can be accomplished by a decrease in concentration or by a decrease in volume secretion or by a combination of both factors. Table 1 (columns 7 to 9) presents the maximum bicarbonate concentration values following secretin. The effect of Diamox on bicarbonate concentration is not as clear cut as is its effect upon total bicarbonate secretion or upon total volume secretion. In the majority, there is a decrease in the maximum bicarbonate concentration after Diamox. However, even at such high dosage levels as 103 and 121 mg. per kilogram, the bicarbonate maxima may not be affected, as indicated by percentage change values of $\pm$ 1 per cent. A statistical study of the bicarbonate concentrations of individual periods indicates that, in essence, Diamox does not affect the bicarbonate concentration Thus, the mechanism of reduction of total bicarbonate secretion appears to be due entirely to the loss of volume flow. The alteration in secretion obtained with Diamox in the patients with pancreatic disease was the same as observed for bicarbonate responses in the normal patients.

Enzyme Secretion. The effect of Diamox on enzyme secretion is illustrated for amylase in Table 1 (columns 10 to 12). Total amylase secretion is affected like bicarbonate concentration. In the majority, there is less amylase after Diamox than before. However, there may be an increased enzyme secretion even at high dosage levels, viz. +67 per cent at 81 mg. per kilogram. The enzyme concentration figures, although not listed, actually rise after Diamox in accordance with their inverse relationship to volume. The decrease in enzyme observed after Diamox can be explained as due entirely to the diminution in volume. The enzyme changes obtained were the same in patients without pancreatic disease as those seen in patients with pancreatitis.

Summarizing the findings: the effect on the pancreatic responses of the carbonic anhydrase inhibitor, Diamox, administered at low dosage levels (10 to 30 mg. per kilogram) is minimal; at the intermediate ranges (30 to 75 mg per kilogram), there is a definite inhibition of the total volume and total bicarbonate output, and at higher ranges (80 to 120 mg. per kilogram) not only is the response to secretin blocked but the data suggest a suppression of the unstimulated volume and bicarbonate secretion. Amylase secretion in response to secretin is also decreased by Diamox. The effect of the drug on volume, bicarbonate, and enzyme appears to be the same for patients with and without pancreatic disease.

DISCUSSION

The importance of increased ductal pressure in the precipitation of attacks of acute pancreatitis[14, 15] and in the pathogenesis of pain in patients with chronic pancreatitis[7, 9, 16] suggests that a drug capable of diminishing the

basal rate of volume secretion and the volume response to secretin might have great prophylactic and therapeutic value in these conditions. To be of use such a drug must have no undesirable side reactions at therapeutic ranges and must affect the secretion of the diseased pancreas as well as the normal organ. Diamox appears to meet these qualifications. For the first time, a drug is now available which influences pancreatic secretion at the cellular level.[17]

It might be argued that the action of Diamox on bicarbonate secretion is undesirable in patients with pancreatitis. The buffering action of the pancreatic juice is important in such patients for preserving a neutral reaction in the duodenum. This protects against the development of a duodenal ulcer which might result from the unopposed action in the duodenum of acid elaborated in the stomach, a situation which is observed clinically in pa-

Table 2. pH Values of Gastric Drainage Specimens before and after Diamox

DIAGNOSIS	DOSAGE MG/KG	LOWEST PH PRE-DIAMOX	GASTRIC PH VALUES POST-DIAMOX
1. Duodenal ulcer	10	3 5	3 5—3 5—7.0—7 0—7 5
2 Post-cholecystectomy syndrome	10	7 7	7.7—7.7—7.5—7.5—7 5
3 Psychoneurosis	14	1 5	6 5—6 7—7 0—3 5—5 5
4 Post-cholecystectomy syndrome	17	4 5	7.0—5 0—6 5—7.0—7 0
5. Migratory phlebitis	30	1 0	2 5—2 5—2.5—4 0—4 5
6 Abdominal pain, etiology?	31	3 5	7.7—7.5—7.0—7 7—6 5
7. Ulcerative colitis	50	3 5	5 5—6 5—6 5—7.0—5 0
8. Subacute pancreatitis	53	2 0	2 0—2 5—3 5—4 5—5 0
9 Amebiasis	56	5 5	5 5—7 0—7.5—7.5—7 0
10 Chronic pancreatitis	63	5 0	7 0—7 0—6 7—6 5—7 0
11 Chronic cholecystitis	81	1 5	3 5—7.5—7 0—5 0—5 0
12 Chronic pancreatitis	85	7 0	7 5—7 5—7 5—7 5—7.5
13 Post-cholecystectomy syndrome	91	1 5	5 0—6 5—6 5—6 7—6 7
14 Chronic pancreatitis	103	2 0	5 0—7.0—7 0—7 0—7.0
15 Duodenal ulcer	105	1 5	4 5—5 0—6 5—6 5—6 5
16 Psychoneurosis	108	6 5	6 5—8 0—8 0—7 5—7.5
17. Duodenal ulcer	112	1 0	4 0—5 5—7 0—7 5—7.0
18. Psychoneurosis	121	1 0	4 0—5 0—7.0—7 0—7 0

tients with severe chronic pancreatitis and also in patients following radical resection of the pancreas for carcinoma. The buffering action of the pancreatic bicarbonate also prevents excessive stimulation of the pancreas by the endogenous secretin which might be induced by the presence of unneutralized acid in the duodenum.

Table 2 presents the *p*H values of the gastric drainage after Diamox The data indicate that in all instances, even among those patients who prior to

Diamox may be more than compensated by the concomitant diminution of

gastric acid formation. In fact, the suppression of hydrochloric acid secretion, per se, may be a desirable physiologic effect in patients with pancreatitis and has been considered as a therapeutic aim itself, both in medical treatment and in surgery.[9]

The observed results encourage further study of this drug to establish the optimal oral and intravenous dosages. The evaluation of Diamox in the therapy of pancreatitis will not be simple and should be conducted in many clinics.

CONCLUSIONS

1. The gastric and duodenal secretion of 18 patients (14 without pancreatic disease and 4 with pancreatitis) was studied by the double secretin test technique before and after the intravenous administration of the sodium salt of Diamox, a potent carbonic anhydrase inhibitor, in dosage ranging from 10 to 121 mg. per kilogram.

2. Diamox, given intravenously at levels of 50 or more mg. per kilogram, is capable of diminishing not only the total volume response and the total bicarbonate output of the pancreas following secretin but also decreases the basal rate of volume and bicarbonate secretion. The rate of enzyme formation appears to be diminished by Diamox. This effect, however, is probably secondary to the volume changes. The inhibition of secretion induced by Diamox was of the same order of magnitude in patients without pancreatic disease as that observed in patients with pancreatitis.

3 The actions of Diamox on pancreatic flow, on pancreatic enzyme secretion, and on gastric acid formation, suggest that this drug may be of value in the treatment of acute and chronic pancreatitis.

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5. Migratory phlebitis	30	1 0	2.5—2 5—2 5—4 0—4 5
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Thus the loss of bicarbonate secretion from the pancreas following Diamox may be more than compensated by the concomitant diminution of

NUTRITION, BODY FLUIDS, AND METABOLISM

INTRODUCTION

CARL A. MOYER

The manner of feeding the ill has undergone remarkable improvement during the past twenty years. Research performed in surgical laboratories has played a role in elucidating the peculiarities of the nutritional requirements pertinent to cancer, burns, infections, and intestinal obstructions; and this research has served as a base for the betterment of the nutritional practices before and after operations. A superficial evaluation of our present capacity to manage the nutritional and fluid problems obtaining with surgically remedial illnesses could well lead to the conclusion: the field of nutrition as it relates to medicine, and more especially to surgery, has been so well ploughed that little more can be learned by belaboring it further. However, deeper scrutiny renders this conclusion untenable. When the starved person's gastro-intestinal tract cannot serve him, the staying and more especially the alleviation of severe starvation with parenterally given food factors is still remarkably difficult. The provision of sufficient food parenterally to meet the individual's catabolic requirements with only monosaccharides and be given in strongly hypertonic solutions o water. For example, an adult bearing an is intestinal tract is obstructed will require, by virtue of the increased metabolic rate attending the suppuration, from 2000 to 4000 calories to meet his catabolic requirement. To balance this with a mixture of 5 per cent protein digest in a 5 per cent solution of a monosaccharide, and a 10 per cent solution of glucose or fructose would require the giving of five to ten liters of fluid—a grossly excessive mass of fluid. To provide the requisite food in an acceptable mass of fluid, 3000 to 3500 ml., a 15 to 30 per cent solution of monosaccharides and a protein digest needs be used. This can be done safely by placing a fine polyethylene catheter into a large vein such as the subclavian and dropping the solution slowly through it. However, the difficulty of feeding the starved only parenterally would be remarkably reduced should means be developed to give lipids intravenously safely. The mitigation of pyrogenicity has been and is one of the major problems in the making of parenteral fat emulsions. Brown and others have obtained some evidence that pyrogenicity may in part be caused by the larger oil droplets in the emulsion. They employed a nonionic detergent to effect dispersal of the lipid This approach to the manufacture of stable emulsions of fat for parenteral feeding should be adopted with great caution—the *nonionic detergents* [monolaurates (Span 20) and stearates (Tween 60)] *are potent co-carcinogens* * Bevilacqua and associates have made rather disturbing observations —remarkable increments of hepatic and splenic lipid attend the giving of fat emulsions intravenously to dogs. This fat is contained largely in the reticulo-endothelial cells of liver and spleen. As much as 40 per cent of the infused fat could be accounted for in the liver alone. Nomura (1929) and

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The loss of tissue incident to the rise in catabolic rate obtaining for a time after partial gastrectomy can be largely prevented by providing 90 to 120 grams of protein and 2800 to 3000 food-calories (Abbott and associates).

The last two great wars and the threat of the third have and are prescribing search for an innocuous colloidal substance which would function osmotically as do the plasma proteins. Gum acacia was first tried—it discomfited the liver; then came gelatin—it gelled when cold and would not flow through a needle; then oxypolygelatin—the supply was too limited; then PVP—it smudged the liver; now it is dextran!

There is evidence that at least some of the dextrans are pyrogenic, they interfere with the cross-matching of blood of the person receiving them, they promote the oozing of blood from sundered capillaries, and they collect in the cells of the liver and the more especially in the renal tubules of burned animals. However, these adverse effects may be accepted as necessary evils provided that the inclusion of dextrans in the saline solution* materially enhances the effectiveness of the saline in reducing the mortality rate among injured animals and men. Millican, Stolhman, and Mowry (Am. J. Physiol., *170*:173, 1952) found four dextrans in saline to be less effective, and one to be equally or a little more effective than a saline solution in reducing the mortality rate of burned and tourniquet-shocked mice. During the Forum sessions of 1954 Davis presented data that could account for Millican's observation of the lack of superiority of dextran over saline in the treatment of burn shock. He found dextran disappearing from the blood stream of burned anesthetized dogs at a very rapid rate and consequently the dextran did not serve to maintain the intravascular volume of fluid after burning. Dextran given to dogs with distended intestines also left the blood stream very rapidly. However, dextran solution maintained the vascular fluid volume well when given to bled animals. In brief: Dextran leaves the blood stream rapidly when given in the face of a significant increase in capillary permeability (burns, intestinal obstruction, and crushing or tourniquet injury) and consequently is no more effective than a saline solution in the treatment of vascular hypovolemia attendant upon the above injuries. However, it stays in the blood stream well when given after blood alone has been withdrawn from the circulation and consequently is, in a limited sense, an effective agent for the temporary treatment of hemorrhagic shock.

Metcalf and Rousselot made some remarkable observations regarding the relationship of the vascular volume expansion obtained with the giving of dextran and the concentration of plasma proteins. The vascular volume expansion was excellent in individuals having plasma protein concentrations of 7.0 or more grams per cent, and poor at levels of 5 to 6 grams. The relative lack of volume expansion with the lower protein levels could not be correlated with variance of dextran retention—it was retained almost as well in the individuals with the low as it was in those having the high concentrations of plasma proteins.

The effectiveness of dextran as a vascular volume maintainer or expander is more in doubt today than it was a year ago. It now appears that its usefulness, if any, will be restricted to the temporary treatment of shock attributable to hemorrhage. Evidence now available to us indicates that it is no more effective than Ringer's solution for treating the shock of burns, scalds, or contusional trauma.

* The dextran solutions available are saline solutions with added dextrans.

Holt (1935) observed this a number of years ago. Rhoads (Southern Surgical Association meeting, 1954) urged that fat emulsions not be given to individuals having hepatic disease or jaundice Hine and others again found the phospholipid, lecithin, to be hemolytic when given intravenously and although caloric balance was attained with it the dog died and the kidneys were remarkably hurt. Lecithin is unsuitable as a parenteral lipid.

Briefly. As yet there is not enough known regarding the preparation of stable non-pyrogenic fat emulsions and their catabolism within the human being to permit their general use intravenously. More research is needed!

The demonstration by Elman and others that the parenteral administration of fructose combined with a protein hydrolysate effected a greater retention of nitrogen than was obtainable with an isocaloric mixture of glucose and hydrolysate has given rise to a good deal of conjecture as to the reasons for it. Christensen and associates have shown that at least some, if not all, of this phenomenon is relatable to the ease with which relatively non-usable or urinary-wastable complexes of protein hydrolysate and sugar are formed between monosaccharide and amino acids during autoclaving and possibly upon standing at room temperature. These urinary-wastable complexes are apparently more rapidly and readily formed between the
the hydrolysate and fructose
just before giving them prac-
etention between solutions of
glucose and hydrolysate and fructose and hydrolysate.

These observations are remarkably important in that they should stimulate further investigations relating to the specific chemical changes occurring in saccharide solutions during sterilization and storage. Sterilization of D-glucose solutions at 115°C. for twenty minutes destroys about 25 per cent of the sugar Coordination compounds of monosaccharides are readily formed in alkaline media, and dilute acids favor the origin of the furanoid forms of lactones and lactols. *What are the metabolic consequences of introducing these substances into man?*

Christensen and his associates also found that the infusion of glucose or fructose before the protein hydrolysate was associated with minimal wastage of amino acids and peptides and the rate of total excretion of nitrogen was lessened.

The above observations imply that maximal metabolic usefulness of a parenterally given protein hydrolysate can be obtained by first infusing a solution of glucose or fructose and then administering the hydrolysate

Lovelace and Hardy succeeded in their endeavor to secure nitrogen balance in man with parenteral feeding by giving 100 to 110 grams of protein and additional amounts of alcohol and sugar (300 to 500 grams) to provide 1800 to 2400 calories per diem but they did not obtain a positive nitrogen balance with these means

The metabolic disturbances attendant upon trauma (gunshot, burns) and surgical procedures are gradually being further defined. Cholette et al have discovered a significant though transient elevation in serum lipids (primarily the neutral fat) for one to ten hours after an operation. In severely wounded and oliguric soldiers Rosen and Levenson found an increase in the hemic concentrations of five aliphatic and two cyclic amino acids between the fourth and tenth days after injury, and a fall in that of glutamic acid on the third day.

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SERUM LIPID LEVELS FOLLOWING OPERATION*

CLAUDE CHOLETTE, MARY ANN PAYNE, GEORGE N. CORNELL, AND JOHN M. BEAL**

Mobilization of depot fat stores for energy occurs during periods of prolonged starvation Recently, it has become apparent that the catabolic period following operation is also associated with a significant loss of body fat. It seemed possible that these metabolic changes might be reflected in the lipid pattern of the serum Man and associates,[3,4] using a titrimetric method for total lipid determination, have demonstrated a decrease in serum fatty acids, cholesterol and phospholipid on the first and second days following operation. Preliminary studies in this laboratory, using the manometric carbon method for total lipid determination, confirmed some of these findings and extended the period of observation. The more acute changes associated with fat mobilization might be expected to occur earlier in the postoperative course than at the time intervals previously observed.

Six patients, three male and three female, were selected for study. The age range for this group was 29 to 82 years. Of the three males, one underwent subtotal gastrectomy under general anesthesia, one had an inguinal hernia repaired under local anesthesia and the other had an excision of a pilonidal sinus under low spinal anesthesia. In the female group, two patients underwent operative intervention for fracture of the neck of the femur. One of these patients had the insertion of a Smith-Petersen nail, the other had the insertion of a Thompson femoral head prosthesis. In both of these patients the procedures were done under general anesthesia The remaining female patient had a ventral hernia repaired under general anesthesia.

These patients did not receive blood, plasma, or oral feedings during the period of study. A fasting blood sample as a control was obtained from each patient immediately prior to operation The time the patient left the operating room was noted and a blood sample was obtained at 1, 3, 5, 7 to 10, and 14 to 24 hours following the removal of the patient to the recovery room Complete lipid patterns were then determined in duplicate on each sample.

METHODS

Total lipid was determined by the carbon manometric method of Ahrens, Eder and Van Slyke[1] The lipid was precipitated by sodium tungstate, washed with dilute sulfuric acid and then dissolved in a 3:1 alcoholic ether mixture (Bloor's solution). Aliquots of the solution were pipetted into Van Slyke combustion tubes, evaporated to dryness and the carbon content determined on the Van Slyke manometric apparatus The milligram per cent carbon was then converted to milligrams per cent total lipid using the conversion factor determined by Ahrens et al Cholesterol was determined by Sperry and Brand's modification[6] of the method of Schoenheimer and

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Sperry,[5] using the classic Liebermann Burchard reagent. Phospholipid was determined by the method of Fiske and Subbarow.

RESULTS

The results of the total lipid determinations are shown in Figure 1. The preoperative value for each patient was considered as zero. Each subsequent determination was plotted as a plus or minus deviation from this baseline.

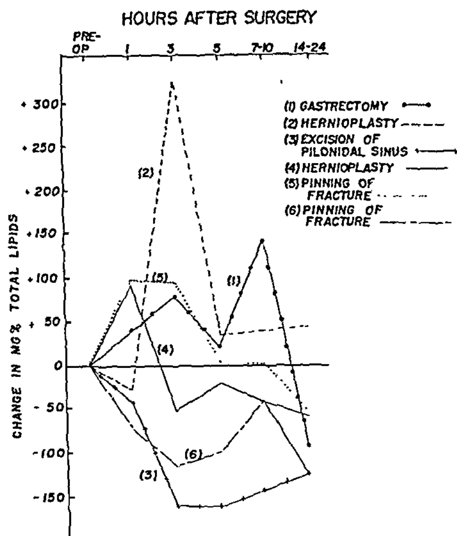


Fig. 1. Postoperative changes of total serum lipids.

The abscissa denotes time in hours after the completion of the operation (1, 3, 5, 7 to 10, and 14 to 24 hours respectively). In four of the six patients there was an initial rise occurring between 1 and 3 hours following the operation. In each this rise was promptly followed in the next period by a significant fall. In three of these four patients the 24 hour values were lower than their control preoperative levels. In patient No. 1, a second rise of serum lipid occurred between the 5 hour determination and the 7 to 10 hour determination. This patient received intravenously injected hypertonic glucose and protein hydrolysate solutions throughout the period of study.

In the remaining two patients (Nos 3 and 6), the total serum lipid values fell sharply over the two periods of observation. In both patients the 24 hour value remained below the preoperative levels.

Figure 2 is a composite picture of the algebraic sum of the total lipid changes which occurred in the entire group of patients in each of the experimental periods Again, the baseline represents the preoperative control values. There is a substantial increase in total circulating lipid during the first two periods of observation This is followed by a decrease of total lipid persisting throughout the 24 hour period.

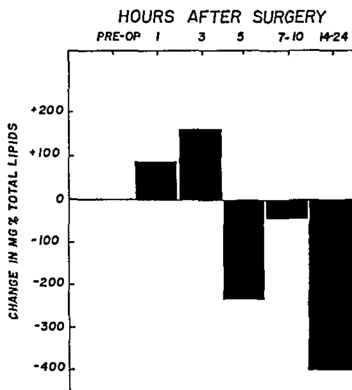


Fig. 2 Postoperative changes of total serum lipids, composite picture.

Additional studies were obtained on five patients who were subjected to cholecystectomy. Determinations were made at 10 and 14 to 48 hours and 1 week intervals following operation. In three of these patients the serum lipid level was persistently below the preoperative value. In the remaining two patients studied at these intervals the serum lipid concentration was elevated above the control level at 10 hours. In each case, the value obtained at the end of 1 week was found to approximate the preoperative level.

In addition to total lipid, cholesterol and phospholipid fractions were determined on each sample. The records of two representative patients are shown in Tables 1 and 2. Patient No. 2 sustained a marked rise in his total lipid. As may be seen in Table 1, this rise in lipid was not associated with any significant change in the cholesterol and phospholipid fractions. In patient No. 3 the total lipid fell consistently throughout the early postoperative period. This fall again was not associated with any significant change in the other two fractions. The neutral fat reflects the change in total lipid,

as this value is derived by subtraction of cholesterol and phospholipid from total lipid. Because the cholesterol and phospholipid fractions remain constant, the changes observed in the total lipid cannot be considered secondary to changes in hydration.

DISCUSSION

It is apparent from these data that alterations in serum lipid do occur in the immediate postoperative period. The present studies indicate that these

Table 1. Postoperative Lipid Changes (Patient No. 2: Hernioplasty, General Anesthesia)

TIME HRS.	TOTAL LIPID MG. %	TOTAL CHOLESTEROL MG. %	PHOSPHOLIPID MG %	NEUTRAL FAT MG. %
Preop.	690	270	249	171
Postop.				
1	663	238	218	207
3	1009	232	233	544
5	725	240	239	246
14–24	735	232	239	264

Table 2. Postoperative Lipid Changes (Patient No. 3: Excision of Pilonidal Sinus, Spinal Anesthesia)

TIME HRS.	TOTAL LIPID MG %	TOTAL CHOLESTEROL MG. %	PHOSPHOLIPID MG. %	NEUTRAL FAT MG. %
Preop.	747	238	236	273
Postop.				
1	705	238	244	223
3	587	214	238	135
5	587	198	265	124
14–24	625	182	210	233

changes occur earlier than previously reported,[3] and are limited to the neutral fat fraction of the serum. The limited data prevent the formulation of any definite conclusion. However, the changes reported in this paper occur at a time in the period following operation when certain other metabolic phenomena occur. During the postoperative catabolic phase an increased caloric requirement must be met, and body fat stores appear to be mobilized. It is possible that the initial increase in serum lipid which was demonstrated in some patients reflects mobilization of depot fat. The exact time of this mobilization may vary from patient to patient but may be an early part of the stress response to operative trauma. The decrease seen later in the postoperative period may be largely the result of starvation The present studies indicate that serum lipid levels may remain depressed for several days following operation.

SUMMARY

Preliminary studies on alteration in serum lipid in the 24 hour period following operation have been presented. Our studies thus far indicate that an early alteration in serum lipid may be a part of the reaction to operation.

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AN INTRAVENOUS FAT EMULSION CONCENTRATE*

Demonstration of Compatibility and Low Order of Toxicity

CHARLES E. BROWN, ARNOLD G. WARE, RICHARD C. SLANKER, AND H. H. ZINSSER**

The search for a satisfactory intravenous fat preparation has been intensified in the past decade as the superiority of fat to protein and carbohydrate as a high caloric source per unit volume of parenteral fluid is evident. The ideal parenteral fat must be metabolized readily and must be free from untoward reactions. It is logical to assume that the physical and chemical characteristics of such a suspension will parallel chyle. Chyle has an average particle size of one micron or less. Numerous reports have established that a variety of fats including sesame oil, corn oil (Mazola), olive oil, coconut oil and mineral oil are utilized in animals and in humans when given intravenously or subcutaneously.[1,2] Emulsions of these oils with an average particle size of one micron or less can be prepared by homogenization with an emulsifying agent. A wide variety of agents, notably the Tweens, gelatin, egg lecithin, soybean phosphatides, monoglycerides, bile salts, cerebrosides and Triton A-20 have been used.[1,3,4] Various investigators have successfully administered intravenous fat emulsions to humans with minimal reactions,[5,6] however, the absence to date of a readily available commercial fat preparation for general clinical use suggests that the goal of the ideal parenteral fat emulsion has not yet been attained. Lack of chemical and physical stability upon storage and transport, inconstancy of thermogenic properties and a significant incidence of side effects such as headache, nausea, vomiting and anemia are problems that must be solved. The advent of nonionic surface active agents prompted reinvestigation of certain facets of the intravenous fat problem. This study was designed to partially evaluate

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the compatibility and toxicity of an intravenous fat emulsion prepared using a nonionic surface active agent as an emulsifier.

PROPERTIES OF THE EMULSION

The Pluronics* are recently developed surface active agents with molecular weights from 2000 to 8000, these weights being unusually high for surfactants. Pluronic F68 with a molecular weight of 8000 was used in this emulsion. No toxic effects were demonstrated in healthy mongrel dogs upon intravenous administration of amounts of F68 equivalent to those amounts used in emulsion preparation.

The emulsion was homogenized at a pressure of 2500 lbs. per sq. inch in a dairy homogenizer which had been thoroughly cleaned and rinsed with pyrogen-free distilled water. One batch was autoclaved at 15 lbs. pressure at 250°F. for fifteen minutes; a portion of the first batch was then filtered through a Berkfeld type filter using an asbestos type filter pad and re-autoclaved for an additional four hours. Particle sizes were determined with an ocular micrometer attached to a darkfield microscope and averaged 0.2 to 0.5 micra with a maximum of one micron. Earlier samples homogenized

Table 1. Composition of Emulsion

Sesame oil	40 grams
Ethyl alcohol	4 grams
Emulsifier	2.5 grams
1/6 M Sodium lactate q s a d.	100 grams

at lower pressures showed larger particle sizes. The samples were stored at room temperature in a lighted room for varying periods, the maximum time of storage being 8 months. Samples remained physically stable and with constant particle size throughout storage and during railway transport over 2000 miles. Addition of dextrose, normal saline, or gelatin did not affect the stability of the emulsion.

Injection of 1 cc. per kilogram of the first batch of 40 per cent emulsion intravenously into rabbits weighing 4 kg. caused an average maximum temperature rise of 1.23°F. When diluted with 19 invert sugar one to four and given to rabbits in a dosage of 10 ml. per kilogram, the average maximum temperature rise was 0.73°F. Injection of the second batch into seven rabbits showed an average maximum rise of 0.35°F.

PROCEDURE

Adult healthy mongrel dogs weighing from 5 to 13 5 kg. were divided into three groups. The 40 per cent fat emulsion was diluted to 10 per cent with ⅙ M sodium lactate for administration to first two groups. Group 1, the acute group, was given single infusions of 10 per cent fat emulsion, then sacrificed at intervals from 0 to 48 hours after completion of infusion The second group, the chronic dogs, was given intravenous fat three times per week and sacrificed at appropriate intervals Several dogs in each of the two groups were heavily sedated with Nembutal while the remainder had no sedation. Several dogs in either group were infused after a fat-free meal while the remainder had fasted. Sixteen dogs were given a total of one

* Pluronics were supplied by Wyandotte

hundred infusions. Amounts of fat infused varied from 1.1 grams to 4 grams per kilo. Infusion rates varied from 0.17 grams (1.7 cc. of 10 per cent emulsion) per minute, to 1.8 grams (18 cc. of 10 per cent emulsion) per minute. Acute dogs were carefully observed for toxic symptoms. Rates of clearing of lipemia, intravascular particle sizes, gross hemolysis and rectal temperature changes were recorded. Acute dogs were followed to time of sacrifice or for 24 hours. An evaluation of body weight changes in addition to the above mentioned data was made on the chronic group of dogs. One chronic dog was given fat intravenously intermittently over a period of 20 months without demonstrable ill effects.

A third group of five dogs was given large quantities of 40 per cent fat emulsion to determine an approximate lethal dose. Total dosage of from 60 to 80 grams or 11.4 grams per kilogram were administered at variable rates of 0.55 gram per minute to 1.3 grams per minute.

Intravenous infusions were given in accessible veins of hindlimbs or forelimbs using plastic tubing.

Complete autopsies including central nervous system examinations were done on all dogs sacrificed or dying, and histologic sections were stained to demonstrate fat with Sudan III.

RESULTS

Eighty-three per cent of injections in acute and chronic dogs were unaccompanied by any reactions suggesting toxicity, however, pain in limb at site of injection, generalized tremors, convulsions, retching, vomiting, tearing, or bleeding from rectum, bladder or oral cavity occurred in 17 per cent of the infusions. All of the aforementioned symptoms except bleeding occurred within one to three minutes after starting the infusions, lasted for a brief period and did not recur during that specific administration Symptoms of bleeding usually occurred late in the infusion or after the infusion had been completed.

The incidence and severity of untoward reactions were lessened by slowing rates of administration

Three dogs were observed to have blood in the stool and one to have blood in the urine Presence of food in the stomach seemed to predispose to vomiting and retching. Gross hemolysis was not observed in the serum of dogs in these acute and chronic groups

Fat levels in blood increased during administration of the emulsions, reached a peak immediately after completion of infusion, then blood lipemia gradually declined with significant amounts of fat remaining in the blood stream for 10 to 15 hours It was observed that particle sizes in the sera within 30 minutes after completion of infusions were smaller and more uniform than in the original emulsion This suggests that larger particles (approaching one micron) were removed first from the peripheral circulation After two to three hours all large particles were absent from the circulation

In dogs heavily sedated with Nembutal, a uniform temperature drop accompanied the administration of the fat emulsion A similar temperature curve was obtained in control dogs sedated with Nembutal and not given fat. In dogs not sedated, an average maximum temperature rise of 1.26°F was observed with extremes from 0° rise to 2.6°F. Individual dogs tended to show a typical thermogenic reaction pattern to the same batch and to different batches of emulsion Temperatures began to rise shortly after

beginning infusions, with a maximum height being reached 2 to 3 hours after completion of infusion. A gradual decline in temperature then occurred with normals being reached in 12 hours. The time of initial temperature decline coincided with the disappearance of larger fat particles from the plasma.

The magnitude of the thermogenic responses could not be correlated to rates of emulsion administration.

Chronic dogs receiving fat emulsion three times per week and fasting on day of administration maintained body weight and in two instances gained weight during the period of study.

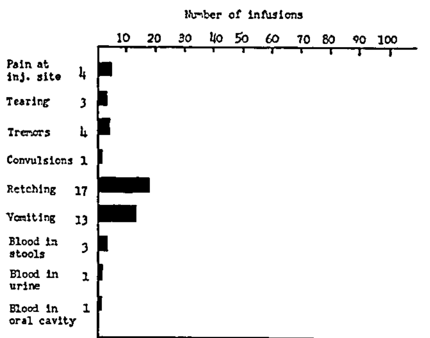


Fig. 1. Frequency of reactions in acute and chronic dogs.

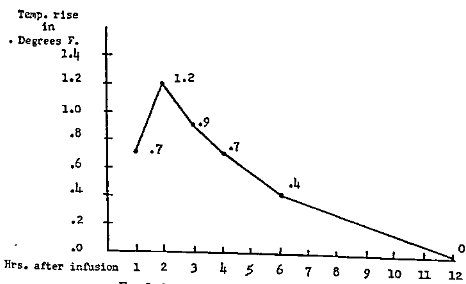


Fig. 2. Average maximum temperatures.

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Fat levels in blood increased during administration of the emulsions, reached a peak immediately after completion of infusion, then blood lipemia gradually declined with significant amounts of fat remaining in the blood stream for 10 to 15 hours. It was observed that particle sizes in the sera within 30 minutes after completion of infusions were smaller and more uniform than in the original emulsion. This suggests that larger particles (approaching one micron) were removed first from the peripheral circulation. After two to three hours all large particles were absent from the circulation.

In dogs heavily sedated with Nembutal, a uniform temperature drop accompanied the administration of the fat emulsion. A similar temperature curve was obtained in control dogs sedated with Nembutal and not given fat. In dogs not sedated, an average maximum temperature rise of 1.26°F. was observed with extremes from 0° rise to 2.6°F. Individual dogs tended to show a typical thermogenic reaction pattern to the same batch and to different batches of emulsion. Temperatures began to rise shortly after

tion. The fat itself was the irritating agent, for a control mixture did not cause pain. The possibility of local vasospasm must be considered here. Tearing, tremors, convulsions, retching, and vomiting may result from small pulmonary emboli or from trapping of fat in the pulmonary circulation. The fact that these reactions occurred within the first few minutes after beginning the administration would seem to support the idea that the larger particles may be trapped in the pulmonary circulation or may coalesce and form emboli. Bleeding from mucous surfaces may result from hemolysis with subsequent anoxia and capillary rupture. The fact that reactions could be lessened by decreasing the initial rate of injection would seem to have clinical significance, for it has been shown that with certain emulsions in humans, the incidence of reactions becomes prohibitive if the fat is given at a rate faster than 10 to 20 grams per hour.[9]

Autopsy established that dogs dying from infusions of 40 per cent fat had pulmonary embolism. Numerous laboratory reports substantiate the production of pulmonary embolism in animals by infusion of intravenous fat.[7, 8] If it is definitely established that large amounts and high concentrations of intravenous fat can produce fatal pulmonary emboli, it may be reasonable to suggest that small, non-fatal pulmonary emboli may be responsible for the inconstant thermogenicity of this emulsion. The fact that earlier emulsions with larger particle sizes gave greater febrile reactions, and the fact that maximum temperature elevations occurred during removal of larger fat particles from the peripheral circulation with this emulsion, would suggest that particle size may affect thermogenicity.

Lambert, Miller and Frost showed that Triton A-20 reduced the rate of removal of infused fat from the circulation.[4] Blood fat levels show that F68 decreased the rate of removal of dispersed sesame oil from the circulation from 3 to 4 hours to 10 to 15 hours. This prolonged level of lipemia may have significance as experiments in progress at present to evaluate the effect of this emulsion in depleted blood volume states, would seem to suggest that fat emulsions prepared with F68 may exert an effect in maintaining blood volumes over significant periods of time. This effect is somewhat unexpected in view of the fact that neutral fat particles should not exert osmotic effect.

Quantities of this emulsion have been administered to healthy human volunteers without untoward effects, and plans are in progress for large scale clinical trials.

SUMMARY AND CONCLUSIONS

A stable fat emulsion of satisfactory particle size for intravenous administration has been prepared using a nonionic surface active agent as an emulsifier.

This emulsion in a 10 per cent concentration has been shown to be compatible with other intravenous alimentations and of low toxicity and thermogenicity in dogs.

Infusion of this emulsion in healthy human volunteers has shown no unfavorable reaction.

Extensive clinical trial of this emulsion is warranted to properly evaluate its effect in humans in health and disease.

Acute dogs sacrificed at intervals of 0 to 48 hours following administration of fat emulsion showed vessels and cells of spleen, liver, adrenals, and glomeruli and tubules of kidneys to be laden with fat. Occasional lung sections showed accumulation of fat. The amount of fat shown in sections decreased as the time interval following completion of infusion increased Chronic dogs sacrificed on the sixth day following their last infusion showed viscera to be both grossly and histologically normal except for occasional cellular infiltration in lungs and increased fat in cells of spleen. No evidence of atherosclerosis or of fat deposition was seen in aortas of dogs receiving prolonged administration of fat

Of five dogs in group 3, four dogs died within 48 hours after receiving

Table 2. Pathologic Findings Typical of Each Group of Dogs

ORGAN	GROUP 1—ACUTE	GROUP 2—CHRONIC	GROUP 3—ACUTE LETHAL
Brain	+ Fat in cerebral vessels*	Normal	+++ Fat in cerebral vessels, petechial hemorrhages
Lung	Cellular infiltration, fat in vessels	+ Cellular infiltration	Pulmonary infarct, pulmonary edema, hemorrhage
Heart	Normal	Normal	Fat in coronary vessels
Liver	++ Fat in vessels in liver cells	Normal	+++ Fat in vessel, in liver cells
Spleen	++ Fat in vessels and cells	+ Fat in cells	+++ Fat in vessels and cells
Kidney	++ Fat in glomeruli and tubules	Normal	+++ Fat in glomeruli and tubules
Bladder	Normal	Normal	Normal
Pancreas	Normal	Normal	Normal
Gallbladder	Normal	Normal	+ Submucosal fat deposit
Gut	+ Fat in vessels	Normal	+++ Fat in vessels, infarct
Adrenal	+++ Fat in cells	Normal	+++ Fat in cells
Aorta	Normal	Normal	Normal

* + = Little, ++ = Moderate, +++ = Marked

the 40 per cent emulsion. All dogs dying showed marked gross hemolysis in serum and had bloody stools. Autopsies of these dogs revealed multiple pulmonary infarcts, pulmonary edema, areas of hemorrhage into lungs, and bloody pleural fluid The brains showed petechial hemorrhages into the white matter Sections of liver, adrenals, and kidney glomeruli and tubules were laden with fat. The intestine of one dog showed a hemorrhagic infarct involving 8 cm. of the small intestine with 200 cc. of bloody fluid in the peritoneal cavity

DISCUSSION

The mechanisms of production of many reactions from intravenous fat are obscure. It was established that pain at the injection site was not due to extravasation outside the vein or to the needle puncture of the vein, but was coincident with the first few drops of fat emulsion to enter the circula-

with 10 per cent glucose; (*d*) with 10 per cent fructose. A partial enzymatic hydrolysate of casein plus pig pancreas (Amigen) obtained from the Mead Johnson Company was used as a sugar-free preparation, as one containing 5 per cent glucose, and as one containing 10 per cent fructose. A partial hydrolysate of bovine plasma proteins (Travamine, Baxter Laboratories, Inc.) was used in sugar-free form and as a solution containing 5 per cent glucose.

The solutions were infused at a uniform rate equivalent to 7.2 ml. of 5 per cent protein hydrolysate per kilogram per hour, maintained by calibrated flowmeters. For the adult male this rate corresponds to slightly over 2 hours for one liter. Comparisons in all cases were made in a single individual over an interval of a few days. Five subjects were normal young persons; ten were convalescent surgical patients with minimal metabolic disturbances.

Pretest urine samples were collected over a 6 to 10 hour period to indicate the background rate of excretion of amino acids, peptides and total nitrogen. The experimental urine sample was collected during the infusion and the succeeding three hours. The analytical techniques and calculations have been described earlier.[3]

RESULTS AND DISCUSSION

The first comparisons were made with fibrin hydrolysates to which sugars had been added by the Abbott Laboratories, prior to final autoclaving. These solutions contained 36 to 40 per cent of their amino acids in peptide form, as indicated in Figure 1. As the figure shows, the loss of amino acids and peptides into the urine increased in the order: sugar-free hydrolysate; 10 per cent fructose; 5 per cent glucose; 10 per cent glucose. In the latter case from 28 to 40 per cent of the peptides infused were lost into the urine during the infusion. In the absence of sugar, however, the peptide wastage was scarcely greater than the loss of free amino acids.

Simultaneous analyses of the blood plasma[4] have shown that the peptide wastages merely reflect the degree of peptiduria; that is, the *peptides* are rejected by the tissues in general, and not by the renal tubular cells specifically. In contrast the levels of the free amino acids in the plasma fall in the opposite order, the highest levels occurring with no sugar, next with fructose, and the lowest with the 10 per cent glucose preparation. Therefore the increased loss of *free* amino acids appears to have mainly a renal origin.

The presence of glucose at 5 per cent in samples of the commercial partial hydrolysate of bovine plasma proteins also intensified the peptidemia and the urinary loss of peptides, the loss being 32 per cent without glucose and 42 per cent with glucose in both of two experiments. In this case the loss of peptides remained substantial even in the absence of sugar; accordingly our earlier conclusions as to the inferiority of peptides to amino acids in nutrient solutions,[3, 5] although modified, are not entirely eliminated by the present experiments.

These increases in urinary wastage with hydrolysates containing sugars had nothing to do with the increased urine volumes sometimes produced by them (especially by glucose at 10 per cent) since insignificant changes were produced in the excretion rates when the urine volume was increased by supplying extra water in association with the infusions.[4]

Hydrolysate-Sugar Interaction during Autoclaving. The unavailability of

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EFFECTS OF SIMULTANEOUS OR PREVIOUS INFUSION OF SUGARS ON THE UTILIZATION OF INFUSED AMINO ACIDS AND PEPTIDES*

HALVOR N. CHRISTENSEN, PATRICIA BRYAN WILBER, BARBABA A. COYNE, AND JOHN HERBERT FISHER

Recent discussions[1] of the relative merits of fructose and glucose as calorie sources in connection with parenteral amino acid nutrition have stimulated us to compare the effects of these two. We have found that the presence of *either sugar in commercial protein hydrolysates interferes with the tissue* utilization of the contained amino acids, particularly those in peptide form. This interference is more serious with glucose. The deleterious effect of the sugars was avoided if they were added to the hydrolysate just before infusion, or if they were infused in advance of the hydrolysate. The latter order of presentation was found in addition to reduce substantially the immediate increase of amino acid catabolism which otherwise occurs. A single examination of the reverse order of infusion (i e., hydrolysate in the forenoon; glucose in the afternoon) has been reported by Elman[2] to give unfavorable results

EXPERIMENTAL

A partial hydrolysate of fibrin (Aminosol) was supplied by the Abbott Laboratories (*a*) with no sugar present; (*b*) with 5 per cent glucose, (*c*)

* From the Department of Biochemistry and Nutrition, Tufts College Medical School, and the Department of Surgery, New England Center Hospital, Boston This investigation was supported in part by a grant (C-1268) from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service, and by a grant from the Abbott Laboratories, Inc Additional details related to this investigation will be published elsewhere

containing residues derived from the sugar and amino acid in equimolar proportion. The sugar is at a dehydration stage corresponding approximately to hydroxymethylfurfural, whereas the amino acid residue is extensively decarboxylated.[8] Our results indicate that in the present instance a very small part of the amino acids had reacted; accordingly, very small

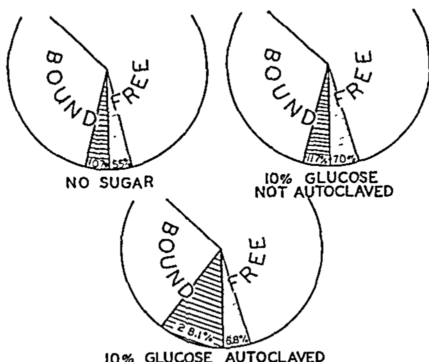


Fig. 2. Effect of glucose sterilely added, and of glucose autoclaved with the hydrolysate solution, on the urinary loss of amino acids and peptides. Autoclaving, 10 minutes at 10 pounds under a carbon dioxide atmosphere. Same method of designation as in Figure 1.

quantities of interaction products must be capable of interfering with the utilization of large amounts of peptides.

The wastage of *free* amino acids, in contrast, was increased in the presence of 10 per cent glucose (Fig. 2) whether the solution was autoclaved or not.*

Decreased Immediate Catabolism of Amino Acids When Sugars Are Infused First. In an attempt to avoid the interference resulting from the presence of sugar in commercial hydrolysates, prior infusion of the sugar solution was tested Ten per cent solutions were infused, also at 7.2 ml. per kilogram per hour, followed immediately by the hydrolysate solution. Under

* A brief note in the Polish literature,[9] apparently preliminary, has been brought to our attention by Dr. R K Richards, of the Abbott Laboratories during the
of this manuscript. According to this report, upon the
bovine blood proteins into normal subjects receiving
and carbohydrate by mouth, the "unutilized amino n
the hydrolysate without glucose, and 18 per cent for the same solution enriched with glucose. Although the analytical procedures are not indicated, presumably the term "unutilized amino nitrogen" refers to free amino acids *excreted* into the urine. The nitrogen balance indices were not significantly different except when the sugar-free preparation was given after a 3-day period on the sugar-containing hydrolysate. One cannot determine from the brief report whether negligible or extensive browning had occurred in the glucose-containing hydrolysate, or how largely browning was involved in this action of glucose.

the commercial casein-plus-pancreas hydrolysate at a glucose content as high as 10 per cent led us to bring a sugar-free preparation to this strength by sterile addition. To our surprise this addition had little effect upon the wastage of peptides into the urine. This result led us to the idea (also suggested by Dr. Douglas V. Frost, of Abbott Laboratories) that the browning reaction might account for the large losses with the sugar-containing hydrolysates. This possibility was tested as follows:

A given subject was infused on three different days with (*a*) the sugar-

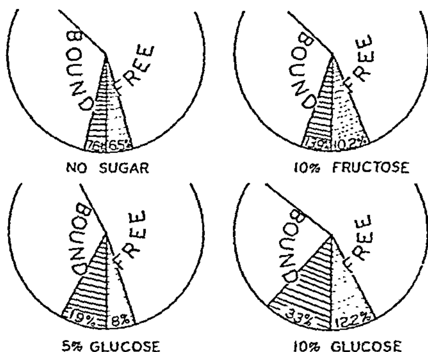


Fig. 1 Increased urinary loss of amino acids and peptides with commercial hydrolysates containing sugars. The preparations were autoclaved after mixing by the Abbott Laboratories The full area of the circle represents the total amino acids infused in each case, with the partition indicated between free and bound (peptide) amino acids. The striated area represents the portion of the bound amino acids lost into the urine, the number indicating the percentage of these bound amino acids lost. The stippled area indicates the portion of the free amino acids lost into the urine, with the number again showing the percentage of this category lost.

free fibrin hydrolysate, (*b*) a fresh mixture of fibrin hydrolysate and glucose to 10 per cent, and (*c*) the same solution autoclaved 10 minutes at 10 pounds under a carbon dioxide atmosphere. The results, illustrated in Figure 2, show clearly that the high wastage arises from products of chemical reaction between the sugar and components of the hydrolysate.

Under the above conditions of autoclaving these interactions are not at [illegible] no perceptible change was found in the amounts of free and [illegible] ation curve of the [illegible] he highly complex [illegible] with the glucose would be expected to show profoundly modified titration behavior. The browning reaction appears to begin with reaction of a sugar derivative with the amino group; extensive condensation occurs to form polymeric products

drolysates of protein increases substantially the wastage of peptides and amino acids during and after intravenous infusion.

2. The extra peptide wastage is avoided if glucose is added sterilely immediately before infusion, but reappears upon autoclaving under usual conditions, and therefore is attributed to products of interaction between sugar and hydrolysate.

3. The prior infusion of sugar substantially decreases the immediate catabolism of amino acids consequent to infusion of a hydrolysate. Sugars present in a commercial hydrolysate had little effect in this respect.

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SURGICAL NUTRITION*

I. Intravenous vs. Oral Route: Nitrogen Balance. A Preliminary Report

JOHN R. LOVELACE AND JAMES D. HARDY

The purpose of this study was to compare the nutritional efficacy of intravenously administered protein hydrolysate-glucose-alcohol solutions with that of identical solutions given by gastric tube, and to compare these results with those obtained with an approximately isocaloric, isonitrogenous whole-protein gastrostomy feeding mixture.

* From the Department of Surgery and Surgical Laboratories, Medical College of The University of Tennessee, and The John Gaston Hospital, Memphis. This work was done under Army Contract No DA-49-007-MD-296.

these conditions no increase whatever occurred in the urinary loss of amino acids and peptides.

Ordinarily when a hydrolysate was infused, there was a large immediate increase in the hourly rate of nitrogen excretion. Since this nitrogen was largely urea plus ammonia nitrogen, an increased catabolism of amino acids was indicated. If a sugar was included in the hydrolysate, this extra nitrogen excretion was scarcely modified, but it was greatly decreased if either fructose or glucose was given in advance of the hydrolysate (Fig. 3). Analyses over an additional four hour period did not modify this conclusion.

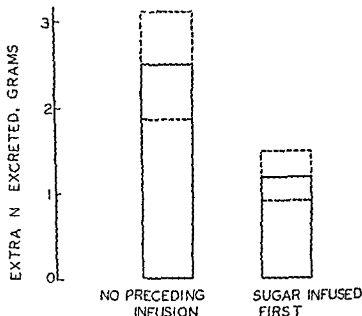


Fig 3. Decreased immediate nitrogen catabolism when a sugar infusion preceded the hydrolysate The bars represent the average extra nitrogen excretion during a 5 hour period

Implications for Intravenous Nutrition. If one considers that calories are not easily supplied in quantity sufficient to render intravenous amino acid nutrition effective, probably both *prior* and *simultaneous* infusion of sugar should be recommended The present results indicate, however, that the simultaneously infused portion probably should not be autoclaved with

'he same solution, since

ere is no evidence that

contain peptides. Advantage may be taken of the lower reactivity of fructose in the browning reaction where autoclaving of sugar and hydrolysate together seems unavoidable. Whether fructose has any advantage over glucose when autoclaving is avoided, and when the sugar infusion is started in advance of the hydrolysate infusion, is not yet clear.

SUMMARY

1. The presence of either fructose or glucose in commercial partial hy-

drolysates of protein increases substantially the wastage of peptides and amino acids during and after intravenous infusion.

2. The extra peptide wastage is avoided if glucose is added sterilely immediately before infusion, but reappears upon autoclaving under usual conditions, and therefore is attributed to products of interaction between sugar and hydrolysate.

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SURGICAL NUTRITION*

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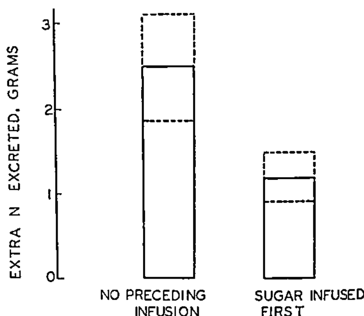


Fig 3. Decreased immediate nitrogen catabolism when a sugar infusion preceded the

5 values by the second The difference is significant at a P value well below 0 001.

Implications for Intravenous Nutrition. If one considers that calories are not easily supplied in quantity sufficient to render intravenous amino acid nutrition effective, probably both *prior* and *simultaneous* infusion of sugar should be recommended. The present results indicate, however, that the simultaneously infused portion probably should not be autoclaved with the solution; perhaps it should not even be stored in the same solution, since the browning reaction also occurs during storage. There is no evidence that this reservation applies to hydrolysates which do not contain peptides. Advantage may be taken of the lower reactivity of fructose in the browning reaction where autoclaving of sugar and hydrolysate together seems unavoidable. Whether fructose has any advantage over glucose when autoclaving is avoided, and when the sugar infusion is started in advance of the hydrolysate infusion, is not yet clear.

SUMMARY

1. The presence of either fructose or glucose in commercial partial hy-

reliable. Nevertheless, the data for the one six-day period are included because of certain considerations which will be discussed below.

During one six-day period the protein hydrolysate-glucose-alcohol and vitamin supplements were given intravenously. In a second period identical fluids were administered by polyethylene gastric tube. During a third period a whole-protein formula* of approximately equal nitrogenous and caloric

DAILY NITROGEN BALANCES

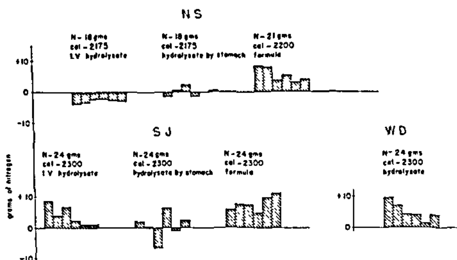


FIG. 2 *N.S.*. This 59 year old female received two liters of Amigen–5 per cent dextrose–5 per cent alcohol, one liter of Amigen–5 per cent dextrose, and one liter of 10 per cent dextrose in water daily during study periods one and two. The three liters of protein hydrolysate were inadequate to produce a positive nitrogen balance with only 2175 calories when given by vein, and barely adequate to produce a nitrogen balance when given by stomach tube The whole-protein formula, in contrast, did produce a definitely positive nitrogen balance.

S J: This 56 year old male received during the first two periods two liters of Amigen–5 per cent dextrose–5 per cent alcohol, one liter of Amigen–5 per cent dextrose, and one liter of Amigen–10 per cent dextrose—an intake which provided both more [illegible] balance was positive [illegible] positive as following [illegible] identical with that received by S J. during the one week of the study, which then had to be discontinued. Note, however, the gradual decline in nitrogen retention following intravenous protein hydrolysate in both W D. and S J. (see text).

content was fed by stomach tube. And, when a fourth six-day period was employed, the whole-protein formula administered by stomach tube was sharply increased (A.P. and G.P.).

No strict sequence of administration of the different fluids was adhered to in the first two patients. This was due in part to the fact that only one feeding pump was available, but it was also felt that apparent differences between the different routes and between the different nutritive substances would merit more serious regard if they persisted under different physiologic

* Homogenized milk, 500 cc., eggs, 4; Gevral (Lederle), 205 Gm.; glucose (Cartose), 10 oz.

METHODS

Seven subjects with proven carcinoma of the esophagus but without known renal or cardiovascular disease were placed in private rooms and given a daily intake which provided from 1800 to 2300 calories and from 18 to 24 grams of nitrogen (Fig. 1-3). In one instance the "whole-protein" formula was doubled in order to examine the effect of this increase upon

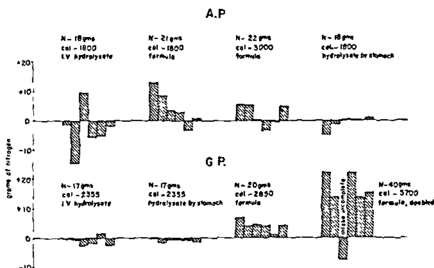


Fig 1 *A P* This 77 year old female received daily during the first and fourth periods two liters of Aminosol, one liter of Amigen with 5 per cent dextrose, and one liter of 5 per cent dextrose The reason for the wide variations in the amount of nitrogen retained during the period of intravenous alimentation was not entirely clear Nevertheless, there was rather little net difference between the nitrogen balance achieved by the intravenous as compared with the oral (gastric tube) administration of identical protein hydrolysate mixtures. *The whole-protein formula produced a definitely positive nitrogen* balance, and the increase in the caloric intake during the third period appeared to have little effect

G P: This 62 year old male received during the first two study periods two liters of Travamin-alcohol, one liter of Travamin-dextrose, and one liter of 10 per cent dextrose in water, providing 2355 calories and 17 grams of nitrogen Note that there was no decided advantage in giving the protein hydrolysate mixtures by vein as compared with the gastric route However, the administration of the whole-protein formula providing only a slight increase in calories and nitrogen was accompanied by a definite retention of nitrogen. Finally, doubling the whole-protein formula produced a markedly positive nitrogen balance.

nitrogen balance (G P., Fig 1) Four consecutive and uninterrupted six-day study periods were completed in two patients (A.P. and G.P.), and three study periods were completed in the other four patients (N.S., S.J., *Fig.* 2, A.V., P.H., *Fig.* 3). *A seventh patient* (W.D., *Fig.* 2) *received the hy*drolysate-glucose-alcohol mixture intravenously for the first six-day period, but thereafter the study had to be discontinued because of his large tracheo-esophageal fistula which rendered the nitrogen balance measurements un-

All excreta were analyzed for nitrogen content, as was all intake. Fat, sodium, potassium, and chloride balances, as well as variations in the body fluid compartments, were also measured. However, space precludes the proper presentation of these data in this report. It is worth noting here, nevertheless, that commercial protein hydrolysates vary greatly in their electrolyte composition, some of them being almost free of potassium. For this reason, supplements of potassium are usually indicated during periods of liquid alimentation.

RESULTS

The nitrogen balances are presented graphically in Figures 1 to 3. It was necessary to administer four liters of protein hydrolysate to produce a consistently positive nitrogen balance.

Intravenous vs. Oral Administration of Protein Hydrolysate. It may be seen that it usually made little difference whether the protein hydrolysate was administered by the intravenous or the oral route, for approximately the same amount of nitrogen was retained in the body in either case, with the exception of patient P.H. Notwithstanding, there were important differences with respect to the route by which nitrogen was excreted under the different circumstances. When the protein hydrolysate-glucose-alcohol mixtures were administered by vein, there was a heavy loss of nitrogen in the urine. On the other hand, when the material was given by gastric tube there was a heavy nitrogen loss in the feces due to frequent stools, the causes of which may be several[1, 2] Yet, the net result was to achieve very similar final nitrogen balances with protein hydrolysate by the two routes.

In three of the seven patients whose data during the intravenous administration of protein hydrolysate are given, there was a progressive decline in the degree of positive nitrogen balance over the study period (patients S.J., N.S., and P.H.). This was not due to a decline in urine volume. Could it be that the body was soon saturated with these protein derivatives and thereafter rejected more and more of this somewhat artificial or incomplete nutritive material? Of course, it would have been desirable to prolong each six-day study to at least ten days, but the exigencies of the clinical situation dictated otherwise.

Oral Protein Hydrolysate vs. Isonitrogenous, Isocaloric Whole-Protein Materials. While in the first three patients studied (A.P, G.P., and N.S.) there was evidence that more of the whole-protein formula was retained in the body, in these instances the formula provided either more calories or more nitrogen, or both, than did the protein hydrolysate-glucose-alcohol materials, rendering rigid comparison open to question. However, in the last three patients (S.J., A.V., and P.H.) the caloric and nitrogenous intake by the two regimens was essentially identical, permitting more precise comparison. Especially in S.J. and A.V. did the whole-protein formula prove biologically superior to the protein hydrolysate mixture. In the case of P.H. the positive nitrogen balance provided by the formula was somewhat more strongly positive than that from oral hydrolysate and sharply superior to that provided by intravenous hydrolysate.

Influence of Clinical Condition of Patient upon Food Utilization. That the cancer patient in the late stages of his disease may be unable to derive discernible benefit from even forced feeding is well known. That the tumor itself is in some way responsible for this physiologic derangement is a reason-

conditions. In the last four patients, however, an identical schedule was rigidly adhered to (Figs. 2 and 3). Moreover, whereas in the patients A.P. and G.P. a variety of protein hydrolysate mixtures was employed (Travamin, Aminosol, and Amigen), in the last four patients only Amigen was used with the glucose and 5 per cent alcohol. Furthermore, while in the first two patients only three liters of protein hydrolysate were given during each 24-hour period, in the last four patients four liters were used. In all instances an aliquot of each mixture administered was analyzed for nitrogen content.

DAILY NITROGEN BALANCES

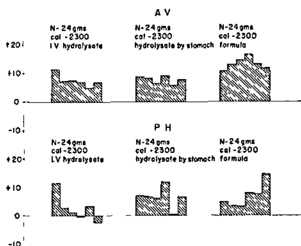


Fig 3 *A V* This 54 year old male received during the first two study periods two liters of Amigen—5 per cent dextrose—5 per cent alcohol, one liter of Amigen—5 per cent dextrose, and one liter of Amigen—10 per cent dextrose The nitrogen balance achieved with the protein hydrolysate-dextrose-alcohol solutions was of similar magnitude in both the intravenous and the oral route However, the positive balance attained with the isocaloric, isonitrogenous whole-protein formula was definitely superior to that of the previous two periods The possible cause of the increased nitrogen retention produced by all three feeding regimens in this subject is discussed in the text

P H This 77 year old male received fluids identical with those given patient A.V. [illegible] protein formula

In this way it was found that, while the estimated nitrogen content of the whole-protein formula was 27 grams, the actual nitrogen content was only 24 grams

In almost all instances the entire volume of nutritive material was infused during the 16 waking [illegible] liter [illegible] in- [illegible] glu- [illegible] of protein hydrolysate-glucose-alcohol in 4 hours, finally, a liter of protein hydrolysate-glucose (10 per cent) in 3 hours. The whole-protein formula was made up in two liters and administered by pump in about 16 hours, though this time varied in different patients. Stools delineating study periods were marked with charcoal or with indigo carmine.

THE EXPERIMENTAL EVALUATION OF PROLONGED INTRAVENOUS THERAPY WITH SOLUTIONS OF GLUCOSE, AMINO ACIDS AND LECITHIN*

DONALD E. HINE AND HAROLD A. HARPER

This is a report of a series of experiments with a 5 per cent lecithin solution† as a source of lipid for intravenous use. The study was designed to answer two major questions: (1) What toxic effects result from the intravenous administration of this preparation of lipid, and (2) can the experimental animal utilize the administered fat and maintain a positive nitrogen balance when it serves as a major source of the caloric intake?

FIRST PHASE: METHODS

Preliminary studies were carried out in 40 adult mongrel dogs, using single intravenous infusions of a solution containing 5 per cent lecithin in 5 per cent glucose or 0 9 per cent saline. All of the animals were in good health and had been fasting for 24 hours prior to the study. Observations in each animal were correlated with the rate of administration of the fat, expressed as grams per kilogram of body weight per hour. These observations included behavior, body temperature and pulse, and the *p*H, coagulation time and concentration of ketones in the blood, as well as the hematocrit.

FIRST PHASE: RESULTS

The results obtained from these studies are summarized as follows:

Behavior. When the solution of lecithin was given at rates exceeding 0.70 to 0.80 grams per kilogram body weight per hour, salivation, lethargy, and muscular twitching were produced uniformly in all animals. Vomiting occurred in some animals. Atropinization of the animal prevented all of these alterations in behavior.

Body Temperature. Alterations in rectal temperature exceeding 0 4°C. were not observed in any of 16 animals studied.

Pulse. The administration of 5 per cent lecithin at rates exceeding 0.70 to 0.80 grams per kilogram per hour uniformly produced slowing of the pulse and "coupling" of the apex impulse in the 10 animals studied. These effects also could be prevented by atropinization.

Blood *p*H. Four of 10 animals exhibited a decline in blood *p*H during the first hour. No correlation between rate of administration and decline in blood *p*H was observed.

Coagulation Time. Observations of the effect of intravenous administration of fat on coagulation time were made on 25 animals Definite prolongation of the coagulation time, as determined by the "three tube method" of Lee-White, was observed whenever the rate of fat infusion exceeded 0 28 to 0.32 grams per kilogram per hour. Reduction of the infusion rate below this range restored normal coagulation time within a few minutes. Figure 1,

* From the Surgical Research Laboratories of the University of California School of Medicine, San Francisco. This study was supported by the Christine Breon Research Fund

† 5% lecithin solution supplied by Cutter Laboratories, Berkeley, Calif.

able assumption With a view to these considerations, it is of interest to note the remarkably positive nitrogen balance which all three feeding regimens produced in patient A.V.—for he was the only one of the patients studied who was in good nutrition and general health at the time of admission Most of the others had lost much weight and were in the late stages of their disease.

SUMMARY AND CONCLUSIONS

1. The nitrogen balance data in seven patients with carcinoma of the esophagus are reported. Six subjects received protein hydrolysate-glucose-alcohol mixtures both intravenously and by gastric tube, and the results by the two routes are compared.

2 In six patients a feeding formula containing whole-protein was administered per gastric tube In three of these the formula was isonitrogenous, isocaloric with the protein hydrolysate regimen, permitting valid comparison. The nitrogen balance produced by intravenous protein hydrolysate was usually similar to that produced by hydrolysate administered by gastric tube. Nevertheless, this was a fortuitous result, for the intravenous route was associated with relatively large nitrogen losses in the urine whereas the gastric route was associated with relatively large losses in the stools.

3 The isocaloric, isonitrogenous whole-protein formula administered by gastric tube produced a more sharply positive nitrogen balance than did the orally or the intravenously administered protein hydrolysate mixture

4. Three liters of protein hydrolysate (18 grams of nitrogen) and a total of 2300 calories barely produced a positive nitrogen balance in most subjects, but four liters (24 grams of nitrogen) usually produced a definitely positive balance.

5. There was some evidence that the one patient who was in an early stage of his malignant disease was better able to utilize effectively both the whole-protein and protein hydrolysate mixtures than were those who had far advanced carcinoma which had produced a marked weight loss.

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tained by daily intravenous infusions of amino acid,* glucose and lecithin solutions. A vitamin preparation† was administered intravenously every other day. The animal also received 300 cc. of normal saline solution, 25 mEq. of potassium and 10 mEq. of calcium intravenously each day. Throughout the study, daily observations were made of behavior, body weight, hematocrit, plasma lipid levels and urinary output. Consecutive 48 hour nitrogen balance determinations were performed. At intervals the levels of plasma protein, electrolytes, and the blood and plasma volumes

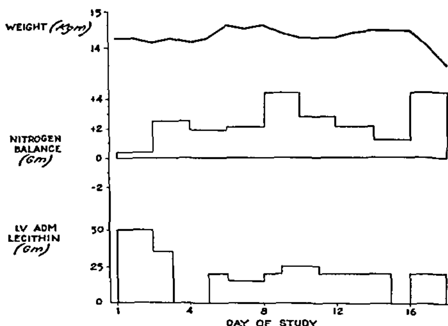


Fig. 2 Alterations in body weight and nitrogen balance resulting from intravenous fat administration.

were obtained. At the end of the study renal and hepatic function were re-evaluated.

SECOND PHASE: RESULTS

The animal survived for 19 days His caloric intake during this period was 660 calories per day except on the fifteenth day, when the intake was 525 calories. Sixty grams of glucose, as a 5 per cent solution, and 60 grams of protein as an amino acid solution were given each day. The daily intake of lecithin solution was varied according to the rate of removal of phospholipid from the plasma.

[illegible] hy through the sixteenth [illegible] or exceeded slightly his [illegible] positive throughout, despite the fact that the lecithin was given intermittently. This fact is explained by the results of daily observations on the rate of removal of the infused fat from the plasma It became obvious quite early that this rate, as calculated from simultaneous plasma volume and lipid phosphorus determina-

* Parenamine, supplied by Winthrop-Stearns, Inc.
† Betasymplex, supplied by Winthrop-Stearns, Inc

illustrates the findings in a typical experimental study. It also indicates that the blood coagulation time is not necessarily altered by the plasma phospholipid level.

Hematocrit. Serial hematocrit determinations were performed on 10 animals during the 72 hour period following an intravenous injection of the lecithin preparation. Alteration of the hematocrit in excess of 2 0 millimeters was not observed in any animal. Extensive hemolysis, as reported by other authors after the administration of various fat preparations, was not encountered in these short-term studies.

Blood Ketone Levels. The concentrations of ketones in the blood were measured in 8 animals. In these experiments the rate of administration of the fat varied from 0 20 to 0 95 grams per kilogram per hour. Only one animal exhibited a rise in blood ketones above the fasting level. Subsequent attempts to reproduce the ketosis in the same animal were unsuccessful.

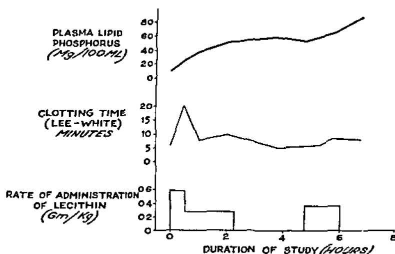


Fig 1 Effect of rate of intravenous fat administration on clotting time and plasma phosphorus level.

SECOND PHASE: METHODS

The second phase of the study was concerned with an evaluation of the effects of prolonged administration of the lecithin solution. For this purpose a healthy, adult male mongrel dog was selected The caloric requirements of the animal were determined during a 6 week period by assay of his daily intake on a standardized kennel ration. It was found that this 15 kilogram dog required 700 to 800 calories per day for maintenance of body weight. His protein intake averaged 3 2 grams per kilogram body weight per day. Prior to the long-term study his renal and hepatic function were studied and measurements were made of the plasma proteins, hematocrit, electrolytes, and blood and plasma volume. Under sodium pentobarbital anesthesia, renal and hepatic biopsies were obtained, and a small polyethylene catheter was inserted into the dog's left jugular vein for subsequent infusions. Clotting within the catheter was prevented by filling the tube with a dilute heparin solution. The animal was then placed in a metabolism cage where he remained throughout the study. He was given nothing by mouth, being main-

the dog gave no evidence of renal damage. It is possible, however, that minor hemolytic episodes were occurring regularly in addition to the obvious episodes of erythrocyte destruction. Such a possibility implies the existence of renal insufficiency long before it was clinically apparent. Nitrogen balance determinations are invalid in the presence of nitrogen retention by the kidney. It seemed desirable, therefore, to place a second dog on the

Table 1.

DETERMINATION	CONTROL	DAY NO. 7	DAY NO. 14	DAY NO 16
Plasma Proteins (Gm %)	7.2	5 8	7.1	....
Electrolytes (mEq /L.)				
Na	151	151	151	151
K	4.8	3.8	4.5	4.1
Cl	109	107	115	95
CO_2	25 0	23.1	20.1	27.7
BSP Test (retention in 15 min.)	1.9%		2.6%	. .
PSP Test (excretion in 2 hrs.)	82%	.	.	50%

same intravenous regimen, but to discontinue the fat infusions after the fourth day while continuing the glucose and amino acid infusions. In this way the effect of the fat on nitrogen balance could be determined before significant hemolysis occurred. For this purpose a 10 kilogram male mongrel dog was prepared as described above. He was given the same intravenous regimen, basing his lecithin intake upon the rate of utilization found in the first animal. The nitrogen balance data obtained in this experiment appear

Table 2.

DAY OF STUDY	LECITHIN (GM)	GLUCOSE (GM.)	PROTEIN (GM.)	NITROGEN INTAKE PER 48 HR. (GM.)	NITROGEN OUTPUT PER 48 HR. (GM.)
1	15.0	45	45		
				13 4	8 6
2	15.0	45	45		
3	15 0	45	45		
				13.4	8 9
4	15 0	45	45		
5		45	45		
				12 6	16.4
6		45	45		

in Table 2. It is apparent that this animal utilized the lecithin as judged by satisfactory maintenance of nitrogen balance during the period when fat was given.

SUMMARY

1 A series of experiments on the intravenous administrations of a lecithin solution to dogs showed that this solution was capable of maintaining body weight and nitrogen balance in these animals.

2. The occurrence of hemolysis was the outstanding toxic effect observed after the continued administration of this lecithin preparation.

tions, was fixed. Consequently, it was possible to omit the fat entirely, as was done during days three and four, and to maintain body weight and nitrogen balance through gradual removal of the accumulated lipid. Figure 2 shows the observations made on body weight and nitrogen balance, and demonstrates the manner in which the fat solution was given. Figure 3 indicates that the rate of removal of phospholipid from the blood, in grams per day, approximated closely the rate of infusion during the fifth to fifteenth day. It was eventually established that this animal was able to remove and utilize a maximum of 20 to 23 grams of lecithin per day. Hemolysis was a major complication in this study. It first appeared following administration of large amounts of lecithin on days one and two The resultant decline in hematocrit is shown in Figure 3. Restoration of hematocrit was accom-

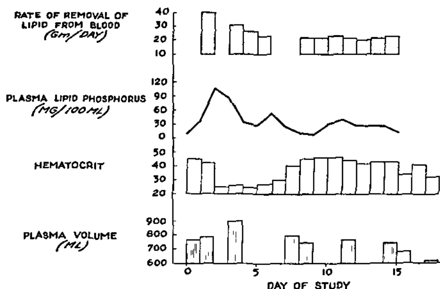


Fig 3 Alterations in rate of removal of plasma lipid phosphorus and lipid phosphorus levels, hematocrit and plasma volumes during 16 to 19 day period.

plished by transfusion and the fat was discontinued during days four and five. Hemolysis as evidenced by falling hematocrit did not again appear until day fifteen It responded only briefly to transfusion on this occasion and was undoubtedly the precipitating factor in the death of the animal, which occurred on day nineteen and was preceded by hematuria, lethargy, vomiting and coma. Postmortem examination revealed an extensive renal lesion consisting of necrosis of tubular epithelium and the presence of many erythrocytes and inflammatory cells within the tubular lumina. Histologic examination of the liver, spleen, lung and pancreas failed to reveal tissue abnormality other than hemosiderin deposits in the liver.* It is significant that no sequestration of fat could be detected within the reticulo-endothelial elements of these organs. Data obtained during the study are shown in Table 1.

Throughout the study, fluid intake was maintained at 1600 cc daily. Through the sixteenth day urinary output averaged 1320 cc. daily with specific gravity ranging from 1.012 to 1.018. Until the terminal epsiode,

* Interpretations of tissue specimens made by Jackson Crane, M D.

Table 1. Average Daily Intake per Kilogram of Body Weight for the First Seven Days Following Operation

PATIENT (NYH#)	CONTROL GROUP I			GROUP II			GROUP III		
	C I (129633)	*C II* (528519)	*C III* (669478)	NKP I (680131)	NKP II (428698)	NKP III (685360)	NCK I (672168)	NCK II (445452)	NCK III (673193)
Nitrogen per kg. (grams)	0.04	0.03	0.06	0.36	0.33	0.38	0.27	0.32	0.46
Calories per kg	7	8	11	22	27	29	39	49	39
Potassium per kg (mEq.)	0.7	0.9	0.6	2.0	1.8	2.4	2.1	2.1	2.1
Phosphorus per kg (mg.)	7.0	5.0	9.0	21.7	30.4	31.4	9.0	17.0	12.3

STUDIES OF CALORIC, NITROGEN AND ELECTROLYTE REQUIREMENTS IN DECREASING POSTOPERATIVE NITROGEN LOSS*

GEORGE N. CORNELL, HELENA GILDER, HENRY MANNIX, JR., AND JOHN M. BEAL**

A previous report has indicated that the catabolic response to major operative procedures can be diminished by markedly increasing the administration of calories, nitrogen and potassium [1] This initial study had included patients who were subjected to either cholecystectomy or partial gastrectomy. Because the response to the degree of trauma which is represented by partial gastrectomy is substantially greater, observations in this group have been extended and form the basis of this paper

METHODS

Nine adult male patients were studie[illegible]
pavilion service. They were from 27 [illegible]
from chronic duodenal ulcer. Routine [illegible]
evidence of renal, cardiac or pulmonary disease present and that their carbohydrate metabolism was normal. All of the patients were on an adequate intake and had lost no weight prior to admission. Each patient received a standard measured adequate diet for a period of four or more days before the operation was performed

The patients were operated upon by surgeons from the resident staff and the length of time required for the operative procedures were reasonably comparable.

The three control patients received infusions of 5 per cent dextrose in saline or distilled water following operation. Two groups of three patients each were supplied with an increased nitrogen intake following partial gastrectomy In one group an increased amount of nitrogen, phosphorus, and potassium was administered without significant augmentation of the caloric supply In the other group a similar increase in nitrogen and electrolytes was supplemented by additional calories in the form of dextrose and alcohol The average daily intake for the seven days following operation is presented in Table 1 and includes the day of operation Two of these patients (C I and NCK I) were also reported in the previous communication.

Standard metabolic methods were employed throughout and have been reported in detail.[1] Balance studies for nitrogen, phosphorus, potassium and sodium metabolism were continued from the time of admission until the patient was discharged from the hospital

Lean wet tissue changes (LTM) have been derived by multiplying the

* [illegible] York [illegible] n the

** [illegible] na R [illegible] Vahey [illegible] staff is also acknowledged.

but an average of 39 calories per kilogram per day was maintained for the eight day period of study. Although the initial rate of loss of lean tissue was approximately the same as in group II it appears that the duration of lean tissue loss was diminished so that he had regained approximately half of the lost tissue protein by the end of the period of observation.

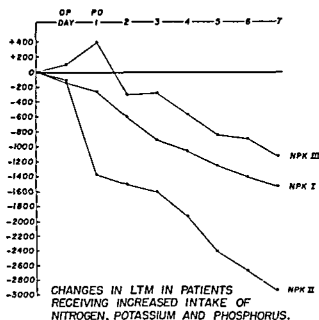


FIG 2. Changes in lean tissue for the three patients in group II receiving an increased nitrogen and electrolyte intake for the operative day and the seven days following operation

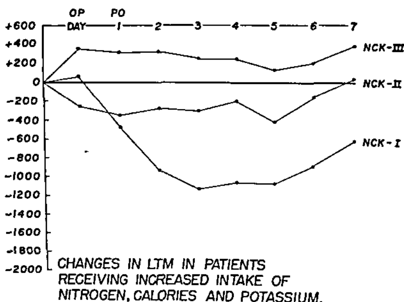


FIG 3 Changes in lean tissue for the three patients in group III receiving an increased nitrogen, caloric and potassium intake for the operative day and the seven days following operation.

nitrogen balance (N) by a factor of 30 (N × 30 = LTM). The lean wet tissue changes have been further converted to apply to a 70 kilogram man to insure a uniform basis for comparison of patients. This was accomplished by dividing the change in lean wet tissue by the daily body weight and multiplying by 70.

RESULTS

The effect of partial gastrectomy on nitrogen metabolism has been charted graphically as changes in lean wet tissue for the three groups of patients in Figures 1–3.

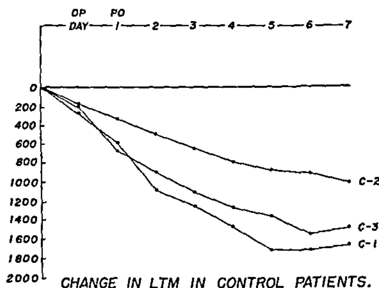


Fig 1. The changes in lean tissue in the three control patients for the day of operation and the seven day period following operation

The catabolic response of the three control patients who received only glucose in the immediate postoperative period extended over a mean period of six days and had a range of five to seven days. The lean tissue loss varied from 138 grams to 208 grams per day for this period and averaged 178 grams per day.

In the second group (group II) the daily nitrogen intake ranged from 0 33 to 0 38 grams per kilogram of body weight per day (Table 1). The caloric and potassium administration in this group also exceeded that of the control study. Nevertheless the caloric intake when calculated on a body weight basis remained far below the minimum daily requirement. In two of these patients the period of lean tissue loss persisted for seven days (Fig 2). The third patient did not lose lean tissue until the second day following operation but for the subsequent period the loss was comparable to that of the other two patients in the group.

The patients who are included in group III require brief individual consideration. The caloric, nitrogen and potassium intake of one (NCK I) approximated the minimum daily requirements of an individual not subjected to trauma. His nitrogen intake was 0 27 grams per kilogram per day, which was lower than that of any other member of group II and group III,

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THE EFFECT OF CAPILLARY PERMEABILITY ON THE MAINTENANCE OF PLASMA VOLUME FOLLOWING THE ADMINISTRATION OF DEXTRAN AND ALBUMIN*

JOHN H. DAVIS, JERREL W. BENSON, MADGE WOLFE, BURT NELSON, AND WILLIAM E. ABBOTT

It has been demonstrated that colloid-containing solutions are effective volume expanders when a deficient blood volume occurs following hemorrhage.[1,2] The use of colloidal solutions has also been widely advocated for patients suffering from a thermal burn. It has been demonstrated, however, that albumin is lost from the circulation in several conditions where increased capillary permeability exists. Since the value of administering colloidal substances under such circumstances has not been demonstrated, this work was undertaken. It is the purpose of this paper to determine the rate of loss of dextran and albumin in hypovolemic patients with and without increased capillary permeability.

METHODS

Eleven mongrel dogs, weighing 15 to 25 kg., were used in this study. Each animal served as its own control, and the experiments were carried out at intervals of two weeks in order to allow the animal time to return to his control state, and to eliminate the administered radioactivity and dextran.

Experiment No. 1—Control. Prior to this and the subsequent experiments the animals were fasted for twelve hours, and on the morning of the experiment they were weighed and blood was drawn for a control (venous) hematocrit. In experiment No. 1 the control hematocrits were obtained and the animals were then given 6 per cent dextran in normal saline solution†

* From the Department of Surgery, Western Reserve University School of Medicine, and University Hospitals of Cleveland, Cleveland, Ohio. This study was supported by grants from the National Institutes of Health, U S Public Health Service (G3408), the Elizabeth Severance Prentiss Foundation, and the Baxter Laboratories, Inc., Morton Grove, Illinois

† The dextran (Gentran) was kindly furnished by the Baxter Laboratories, Morton Grove, Illinois.

The two other patients in group III received an intake of nitrogen and calories that was estimated to be markedly in excess of minimum daily requirements for normal individuals. Figure 3 indicates the extent to which the catabolic phase was altered in these two patients. In one (NCK II) the period of lean tissue loss extended over two days and the total calculated amount of lean tissue lost was approximately one-third of the control group. On this basis it was estimated that the patient had regained his tissue protein loss by the end of the seven day period of observation

The catabolic phase was entirely abolished in the third patient (NCK III). Review of the nitrogen balance data demonstrated that the patient remained essentially in nitrogen equilibrium during the period after operation. Thus while it cannot be inferred from the calculations for deriving lean wet tissue that the patient was actually synthesizing body protein during the postoperative period, it is apparent that the usual catabolic response did not occur and that the patient's tissue protein was not depleted as a result of operative stress.

DISCUSSION

The possibility of diminishing the catabolic response to operation has not been clearly established. It is apparent that moderate increase of nitrogen and caloric intake does not appreciably influence this postoperative phenomenon.[1, 3] The problem is further complicated by certain factors that have been well outlined elsewhere, including the degree of trauma and the nutritional status of the patient.[3] Furthermore, because alimentation of the patient following major operative procedures must be effected by the parenteral route, difficulties are encountered in significantly increasing the caloric and nitrogen intake. Even in the present investigation the methods employed are not feasible for routine application

Evidence has been presented previously that markedly increasing the caloric and nitrogen administration following trauma would result in a nitrogen sparing action and this report is an extension of such investigation.[1, 2, 5] It would appear from the present experiments that increasing only the nitrogen intake is not sufficient to alter the catabolic response to the degree of trauma represented by partial gastrectomy. However, when a significant caloric intake is added, the loss of lean tissue is diminished It is of interest that recent evidence has been presented that a considerable increase in the caloric intake in the absence of a source of exogenous nitrogen failed to alter the characteristic metabolic response to operation.[4] The significance of a simultaneous increase of nitrogen and calories thus becomes apparent.

The effects of variations in the potassium and phosphorus intake have not been sufficiently studied at the present time to warrant any statement.

SUMMARY

Further studies on lean tissue changes in patients who have been subjected to partial gastrectomy have been presented. These investigations indicate that when an adequate intake of nitrogen is supplied the administration of a markedly increased amount of calories will diminish the catabolic response to operative trauma to the degree represented by partial gastrectomy.

dextran and I^{131} labeled albumin is accepted as the volume at zero time, or the time of maximum volume expansion. In the control series of dogs it is known that the plasma volume expansion was approximately 400 ml. since this is the amount of dextran which was injected. At the end of one hour there was no change in the hematocrit or the plasma volume, but 5 per cent of the I^{131} albumin and 12 per cent of the dextran had already disappeared from the circulation. At the end of four hours the plasma volume and

Table I. Percentage Change from Zero Time

		1 HOUR	4 HOURS
Control	Hematocrit	0	0
	Plasma volume	0	0
	I^{131} } % total	95	81
	Dextran } dose	88	70
Dehydration	Hematocrit	0	0
	Plasma volume	+7	+7
	I^{131}	99	83
	Dextran	94	72
Hemorrhage	Hematocrit	−7	0
	Plasma volume	+12	+1
	I^{131}	96	80
	Dextran	93	70
Intestinal distention	Hematocrit	+14	+27
	Plasma volume	−20	−35
	I^{131}	75	50
	Dextran	70	50
Thermal burn	Hematocrit	+16	+57
	Plasma volume	−23	−59
	I^{131}	67	27
	Dextran	61	30

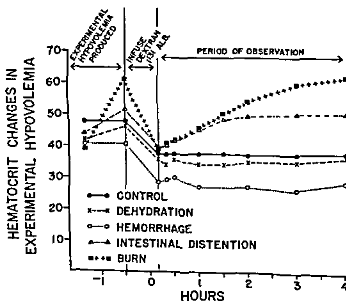


Fig 1. Changes in hematocrit following the infusion of dextran and RIHSA in dogs with hypovolemia produced by different methods.

(20 ml per kilogram of body weight) intravenously. The dextran was administered over a period of 30 minutes and at the end of the injection 20 ml. of radioactive iodinated human serum albumin (RIHSA) were injected intravenously. Blood samples were then drawn from a different vein at 10, 20, 30, 60, 90, 120, 180 and 240 minutes after the injection of the albumin. The 10 minute sample was used to determine the plasma volume directly[3] and the blood volume was calculated from the plasma volume and the hematocrit obtained at this time. Dextran concentration was determined by the method of Metcalf and Rousselot.[4]

Experiment No. 2—Dehydration. The animals were given 5 per cent dextrose in water (20 ml. per kilogram of body weight) intraperitoneally and four hours later the same amount of fluid was withdrawn from the abdomen. Hematocrits were obtained at this time and the animals were given 6 per cent dextran and radioactive albumin intravenously as in experiment No. 1.

Experiment No. 3—Hemorrhage. In these animals an 18 gauge needle was inserted into the femoral artery and the animals were rapidly bled (20 ml per kilogram of body weight) into sterile blood transfusion bottles. The blood was saved and re-infused at the termination of the experiment. Sixty minutes after the hemorrhage, hematocrits were obtained and dextran and radioactive albumin were administered.

Experiment No. 4—Intestinal Distention. In these animals a laparotomy was performed, the intestine was occluded at the junction of the duodenum and jejunum and at the ileocecal valve by means of rubber shod clamps, and a Foley catheter was inserted into the terminal ileum so that the intestine could be distended with air. The incision was closed with stainless steel wire and in several animals an inverted Pyrex bowl was placed in the wound to allow visualization of the distended bowel so that microscopic observation of the changes in the circulation could be made The intestine was then inflated with air to a pressure of 60 mm. Hg for one hour and then released. Thirty minutes after the release hematocrits were obtained and the dextran and radioactive albumin administered as in previous experiments At the end of the experiment the clamps were removed, the abdomen was closed and the animal given intravenous fluids to alleviate the deficits incurred during this procedure

Experiment No. 5—Thermal Burns. The anesthetized animals were burned by immersion in water at 70°C for thirty seconds to a line drawn on the body four inches below the axillary fold Sixty minutes after the burn, hematocrits were obtained and the dextran and radioactive albumin were injected intravenously.

RESULTS

The mean values obtained in each experiment are summarized in Table 1. The percentage changes from the control values obtained at one hour and four hours are plotted, since these time intervals allow a comparison with previously published papers. The changes in the hematocrits are shown in Figure 1 and the disappearance curves of RIHSA and dextran are shown in Figure 2. This paper represents a preliminary report and the objective of this portion of the study was to determine the difference, if any, in the disappearance rates of colloid in various states of hypovolemia. The plasma volume measured at ten minutes after the administration of the

cent of the control value. At this time 50 per cent of the dextran and 50 per cent of the administered albumin had disappeared from the circulation.

In the fifth series of experiments, that of thermal injury, the changes were the most marked of all the experimental groups. At the end of one hour the hematocrit had risen 16 per cent and the plasma volume had fallen 23 per cent. The greatest loss of albumin occurred in this experiment, 33 per cent at the end of one hour; and 39 per cent of the administered dextran had disappeared at this time. At the end of four hours the changes were even more marked in that the hematocrit had risen 57 per cent above the control value and the plasma volume had fallen 59 per cent below the control value. At this point, 73 per cent of the administered albumin and 70 per cent of the administered dextran had disappeared from the circulation.

DISCUSSION

The administration of a colloidal solution to normovolemic animals expands the blood volume and this expansion is maintained for varying periods of time. The rate of excretion of the plasma expander for the most part controls the period of expansion. In our experiments the expanded volume was maintained for the duration of the experiment (four hours). Hammarsten, Heller and Ebert[5] carried out similar experiments in volunteer men and demonstrated that as the dextran disappeared from the circulation the plasma volume was maintained by the addition of endogenous protein. This probably accounts for the maintenance of volume in our animals at the end of four hours even though 30 per cent of the administered dextran had disappeared from the circulation.

Once the effect of dextran had been determined in normal animals, the effect in animals made hypovolemic without increasing the permeability of the capillary membrane was studied. In 1935, Darrow and Yannet[6] summarized the changes which occur in the extracellular fluid and electrolyte following the intraperitoneal administration of 5 per cent dextrose in distilled water. In the present study, four hours after the injection of the 5 per cent dextrose in distilled water, an equal volume of fluid was withdrawn from the peritoneal cavity. Although electrolyte-free solutions had been administered the solution withdrawn contained sodium, potassium, and chloride in a concentration similar to that of the plasma. This shift of extracellular electrolytes into the peritoneal cavity results in a hypotonicity of the extracellular fluid which, in turn, leads to an intracellular shift of water, thus producing hypovolemia. Although a distortion of both extracellular electrolyte and water was produced, the disappearance rate of the administered dextran and iodinated albumin was the same as in the control experiments.

The efficacy of dextran in expanding the blood volume following hemorrhage has been confirmed experimentally and clinically.[1,2,5,7,8] Increased capillary permeability probably does not occur following hemorrhage unless anoxia occurs. In our experiments the plasma volume was expanded and the expansion maintained similar to that in the control animals throughout the four hours of the experiment.

Thus, it can be stated that in normovolemic animals and in animals made hypovolemic without increasing the permeability of the capillary membrane, a colloid will remain in the circulation over a period of time depend-

hematocrit were still maintained, but 19 per cent of the administered albumin and 30 per cent of the administered dextran had disappeared from the circulation

In the dehydration experiments hemoconcentration occurred during the four hours of dehydration, as can be seen from the rise in hematocrit. At one hour after the administration of the dextran and albumin the hematocrit had not changed although the plasma volume had increased 7 per

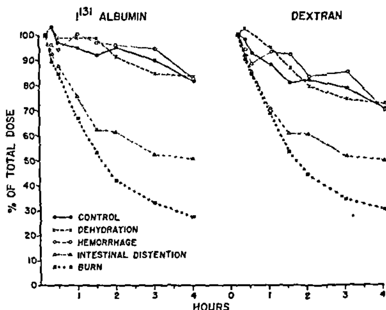


Fig 2 Disappearance of dextran and RIHSA from the blood of dogs with hypovolemia produced by different means

cent above the immediate postinfusion value. At the end of four hours the hematocrit still showed no significant change and the plasma volume was still expanded some 7 per cent above the immediate postinfusion level. Approximately 17 per cent of the administered albumin and 28 per cent of the dextran had disappeared from the circulation.

In the hemorrhage experiments it was noted that one hour after the infusion of dextran the hematocrit had fallen an additional 7 per cent and concomitantly the plasma volume had risen an additional 12 per cent. Over the next three hours there was a gradual rise in the hematocrit and a slight diminution of the plasma volume so that at the end of four hours the levels were again equal to the levels at zero time. Again, there had been a loss of albumin so that 20 per cent had disappeared from the circulation, and a loss of dextran, in that 30 per cent had disappeared from the circulation.

The changes which occurred in the animals undergoing intestinal distention were quite different from those in the previous three experimental groups. One hour after the infusion of dextran the hematocrit had risen 14 per cent and the plasma volume had diminished 20 per cent. In addition, 25 per cent of the administered albumin and 30 per cent of the dextran had disappeared from the circulation. At the end of four hours the hematocrit had risen 27 per cent while the plasma volume had diminished 35 per

SUMMARY AND CONCLUSIONS

Because of the recent emphasis on the use of plasma expanders a study was undertaken to test their value in various types of hypovolemia. From this study the following conclusions seem to be justified:

1. In the normal, dehydrated, and bled animals the use of a colloidal solution, dextran, re-expands the plasma volume and maintains the expansion over the four hours of the experiment.

2. In animals suffering from intestinal distention, or a thermal burn, there is an increase in the permeability of the capillary membrane with a resultant increase in the loss of colloid.

3. The ineffectiveness of colloid-containing solutions in maintaining adequate plasma volume expansion in the face of increased capillary permeability makes their value questionable. It is suggested that further evaluation of electrolyte solutions in treating these states is desirable.

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ent upon its physical characteristics. So long as the colloid remains in the circulation it will exert oncotic activity and tend to hold water

As early as 1907, VanZwalenburg,[9] while studying the circulation in the wall of distended bowel, noted the effusion of a plasma-like material from its surface. Gendel and Fine[10] measured the plasma volume and noted an average diminution of 36 per cent four to six hours after intestinal distention. (Our animals showed a plasma volume deficit of 35 per cent at the end of four hours) Gatch and Battersby[11] determined the protein concentration of the plasma-like material "weeping" from the bowel surface and found that it contained 3 to 4 gm of albumin per 100 ml. A direct loss of colloid (albumin) is demonstrated by these experiments and it would be expected that dextran would also "weep" from distended bowel. Our experiments show this loss by the greatly increased loss of dextran from the circulation (50 per cent at the end of four hours) and by the direct measurement of the concentration of dextran in the fluid which accumulated in the peritoneal cavity. Albumin was lost at a similar rate.

Extensive thermal injury produces perhaps the greatest loss of plasma protein through an abnormally permeable capillary membrane. In 1944, Cope and Moore[12] studied the movement of colloids and electrolytes by means of radioactive dyes and proteins. They demonstrated that the permeability of the capillary membrane produced by thermal trauma allowed colloid to cross the membrane at almost the same rate as electroytes. That such rapid losses occur is clearly demonstrated by the 59 per cent decrease in the plasma volume and the disappearance of 70 per cent of the administered albumin and dextran during the four hours of our experiment.

With the preceding experimental observations in mind, the problem of therapy increases in complexity The generally accepted method of treating hypovolemia by means of colloid-containing solutions is valid only if no increase in capillary membrane permeability exists. When increased capillary permeability has been created the circulating colloid rapidly disappears from the circulation and becomes deposited in the injured area. Two problems must now be considered. (1) Is there any advantage in giving colloid-containing solutions when increased capillary permeability exists? (2) Is the deposition of the colloid in the injured area beneficial or harmful? In answer to the first question some evidence is available to indicate that electrolyte solutions can maintain the circulating blood volume. Most of the experimental evidence has accumulated from the treatment of thermal trauma Various investigators[13-15] have demonstrated that survival rates in burned animals treated with electrolyte solution equal or excel the results of treatment with colloid solution. If the success of therapy depends upon the development of an obligatory edema to a point where further loss ceases then the electrolyte solutions should be effective. Berman[16] has implied that this is true by treating burned animals and patients with subcutaneous saline. The wound itself is infiltrated with exogenous water and electrolyte so that endogenous losses are minimized and circulating blood volume thereby maintained Although final proof of such a concept is lacking the rapid disappearance of colloid from the circulation in conditions of increased capillary permeability should make us re-evaluate the role of colloid solutions in the treatment of such states.

The answer to the second problem remains an enigma of medical research, and further investigation of this problem is necessary.

per kilogram of body weight and containing not more than 4 grams of protein per day. (The high caloric intake was used to rule out the factor of restricted caloric intake and the value of 50 calories per kilogram of body weight was in the range utilized by Rose in establishing minimum oral requirements of the essential amino acids.) Daily vitamin requirements were also administered orally.

The experimental program of each subject consisted of the following:
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Table 1. Daily Intake of Essential Amino Acids at Dosage Level of 10 Grams of Nitrogen (Grams/Day)

AMINO ACID	RECOMMENDED DAILY ORAL INTAKE (ROSE)	SOLUTION A (1 LITER OF 2.5% AMIGEN + ESSENTIAL AMINO NITROGEN)*	SOLUTION B (1 LITER OF 5% AMIGEN + NON-ESSENTIAL AMINO NITROGEN)*	SOLUTION C (1⅓ LITERS OF 5% AMIGEN)*
L-Tryptophane	0.5	0.47	0.6	1.0
L-Phenylalanine	2.2	2.11	2.2	3.7
L-Lysine	1.6	1.52	3.2	5.3
L-Threonine	1.0	0.95	2.0	3.3
L-Valine	1.6	1.52	3.1	5.1
L-Methionine	2.2	2.13	1.0	1.7
L-Leucine	2.2	2.1	3.7	6.2
L-Isoleucine	1.4	1.47	3.1	5.1

*Microbiological analyses.

Depletion period—2 days. Subject given oral diet only.

Test period—3 days. Subject given another of the parenteral solutions at a daily nitrogen level of 10 grams in addition to the oral diet.

Depletion period—2 days. Subject given oral diet only.

Test period—3 days. Subject given third parenteral solution at a daily nitrogen level of 10 grams.

Depletion period—2 days. Subject given oral diet only.

During the three day test periods the parenteral solutions were administered twice daily (in the morning after breakfast and in the afternoon after the noon meal) at the dosage level of 5 grams of nitrogen (10 grams daily) and at an approximate injection rate of 2½ grams of nitrogen per hour. The order of administration of the test solutions was varied from patient to patient. Daily (24 hour) urine collections were made and the daily urinary nitrogen loss was determined using Hoffman's photelometric modification of the Koch and McMeekin direct Nesslerization micro-Kjeldahl method.[6]

The nitrogen balance index method of Allison was used to compare the effectiveness of utilization of the three test solutions.[7,8] This index (K) is calculated from the formula $UN = (I\text{-}K)\ AN + UNo$ where UN is the daily urinary nitrogen (mg./day/kg.), AN is the daily nitrogen intake (mg./day/kg.), and UNo is the excretion of urinary nitrogen on a protein free diet (mg./day/kg.). Allison has demonstrated that the relationship between nitrogen intake and nitrogen balance is linear in the region of low

EVALUATION OF A "BALANCED" AMINO ACID SOLUTION FOR PARENTERAL USE*

TILDEN C. EVERSON AND JOHN F. LAWS**

Although parenteral amino acid and protein solutions are of aid in supplying protein to patients who are unable to take adequate amounts of food orally, an appreciable quantity of the protein furnished by these parenteral solutions is excreted in the urine unchanged (free amino acids and small polypeptides) without being utilized in the building of tissue protein.[1-3] There are several known factors which may impair the efficiency of utilization of parenteral protein solutions in protein synthesis, such as restriction of caloric intake, rate of administration, etc.

A factor which deserves at least theoretical consideration, however, is the possibility that some of the wastage of protein from parenteral protein solutions is due to the fact that the essential amino acids may not be present in the solution in the optimum amounts or optimum ratio for the maximal synthesis of body protein. Thus a factor limiting the effectiveness of a parenteral protein solution might be the low concentration of one or more of the essential amino acids or a lack of the proper ratio of the essential amino acids

METHOD

Three parenteral protein solutions were prepared by alteration of a standard commercially available parenteral protein solution (5 per cent Amigen) †

Solution A The concentration of Amigen per liter was adjusted (reduced to 2 5 per cent) and certain of the essential amino acids added to obtain a solution "balanced" with respect to the recommended daily oral intake for each of the essential amino acids as experimentally established by Rose and co-workers in recent years[4] (Table 1). Glycine, *dl*-alpha alanine and urea were added as sources of non-essential amino nitrogen in quantities sufficient to bring the nitrogen level to 10 grams per liter. The effectiveness of these substances as sources of non-essential amino acids has been established experimentally by growth studies.[5]

Solution B (Control). A liter of 5 per cent Amigen was modified by the addition of glycine, *dl*-alpha alanine and urea in quantities sufficient to raise the nitrogen level to 10 grams per liter

Solution C (Control). Unaltered 5 per cent Amigen was used in a volume sufficient to provide a daily nitrogen level of 10 grams. This required the administration of 1⅓ liters of 5 per cent Amigen.

The relative efficiency of these three solutions was compared by depletion-repletion studies of 20 days duration in each of nine adult human subjects. Each subject was given an oral diet calculated to provide 50 calories

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gratefully acknowledged.

** With the technical assistance of Everett T. Hoppe and Anna Poulos, B S.

† Solutions prepared and supplied by Mead Johnson and Company.

recommended intravenous intake of each of the essential amino acids it may be possible to further improve the theoretical effectiveness of parenteral protein solutions.

SUMMARY

A parenteral protein solution "balanced" with respect to the recommended daily oral intake of each of the essential amino acids was found to be more efficiently utilized than two "unbalanced" control solutions in seven of nine adult human subjects by 20 day depletion-repletion experiments.

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BODY FLUID DYNAMICS. I. HYPERTONIC SALT SOLUTION IN SURGICAL THERAPEUSIS* †

JAMES D. HARDY, JOHN R. LOVELACE, AND HARWELL WILSON

The exploratory investigations of body fluid compartments have been extended across the plasma and the interstitial spaces until now the cell membrane represents the current frontier. Although our knowledge of intracellular fluid equilibrium is still meager, as additional information in this sphere becomes available many perplexing questions may be answered. For instance, what are the factors which influence the "permeability" of the cell membrane to the various electrolytes? How is cell membrane "permeability" affected by variations in cellular metabolism? Are the osmotic equivalents of the several cellular electrolytes altered in disease? To what extent does the cell water content fluctuate in clinical fluid imbalance? What are the

* Department of Surgery and Surgical Laboratories, Medical College of the University of Tennessee, and the John Gaston Hospital, Memphis. This work was done under Army Contract, No. DA-49-007-MD-296

† An abstract of this work was presented before the American Physiological Society.[10]

negative and low positive balance (region of nitrogen equilibrium) and that the slope of the curve (K) represents the rate of change of nitrogen balance with respect to nitrogen intake and thus measures the degree of retention of the nitrogen source by the subject. The nitrogen balance index was calculated for the first and third days of each test solution. In calculating each index the endogenous nitrogen excretion (UNo) of the closest depletion period was used.

RESULTS AND DISCUSSION

The nitrogen balance indices obtained with the three parenteral solutions in the nine subjects studied are summarized in Table 2. The average nitrogen balance index for solution A ("balanced" Amigen) was 0.76 as compared with 0.61 for solution B (Amigen modified by addition of nonessential amino nitrogen) and 0.69 for Solution C (1½ liters of standard Amigen). In seven of the nine subjects the average nitrogen balance was higher for the "balanced" solution than for either of the other two solutions

Table 2 Nitrogen Balance Indices of Parenteral Protein Solutions

SOLUTION	SUBJECTS 1	2	3	4	5	6	7	8	9	AVERAGES
Solution A ("balanced")										
First Day	0 96	0 55	1 08	0 73	0 74	0 74	0 53	0 52	0 55	0 71
Third Day	1 04	81	86	73	95	69	25	98	98	81
Average	1 0	68	97	73	84	72	39	75	77	76
Solution B										
First Day	57	66	88	37	44	98	1 00	27	73	.66
Third Day	76	33	14	69	75	47	52	72	47	57
Average	67	50	61	53	60	73	76	50	60	61
Solution C										
First Day	99	46	91	75	23	93	26	83	.79	69
Third Day	82	57	97	53	43	96	87	43	62	69
Average	91	52	94	64	33	95	57	64	71	69

Considering only the seven subjects in whom there was evidence of superior utilization of the "balanced" solution the average nitrogen balance index for solution A was 0.82 as compared with 0.56 for solution B and 0.68 for solution C

These findings suggest that part of the loss of protein from parenteral protein solutions may be due to the fact that the essential amino acids are not present in the optimum amount or optimum ratio for the most efficient formation of tissue protein Christensen and co-workers have shown that when some acids are injected or fed in high concentration the ability of cells to retain other amino acids is impaired and an inhibitory effect on nitrogen retention occurs [9] Thus nitrogen retention might be adversely affected not only by the small quantity of some essential amino acids but also by the excess quantity of other amino acids.

There is no evidence at the present time to indicate that the daily requirement of each of the essential amino acids is necessarily the same when the amino acids are given intravenously as when they are taken orally. Thus it is possible that with the experimental establishment of the minimum and

felt much improved. Nevertheless, it did not appear reasonable to assume that the marked improvement in the signs and symptoms of extracellular water and *salt* depletion was due entirely to the moderate elevation of the depressed serum sodium and chloride levels, in the absence of significant alterations in extracellular fluid and plasma volumes. To examine this question in a quantitative manner, a liter of 3 per cent sodium chloride solution was administered intravenously to 30 patients, without untoward symptoms. In 17 patients the infusion was completed in precisely one hour, and the results in these subjects form the basis of the present report except where otherwise indicated.

The hypertonic saline infusion increased the extracellular fluid volume an average of 33 per cent and the plasma volume (measured in 7 patients) an average of 30 per cent. These changes were in accord with the Darrow-Yannet concept of body water equilibrium which holds that, since sodium and chloride remain largely extracellular, the infusion of an excess of these ions must result in a movement of water out of the cells to preserve osmolar neutrality.[8]

METHODS

Adults in good general health awaiting elective operations were selected without regard to degrees of obesity. Control measurements of plasma carbon dioxide combining power, chloride, sodium and potassium were mad[illegible] (plasma volume and [illegible] total body water (D_2[illegible] was obtained by subtracting the thiocyanate space from the measured total body water. Red cell sodium and potassium were determined with the Beckman Model B flame spectrophotometer, as were the plasma and urinary concentrations of these ions.

After these preliminary measurements, one liter of 3 per cent sodium chloride solution was infused intravenously in one hour. Promptly at the end of this time blood was drawn for repeat plasma electrolyte measurements, and the plasma and total blood volumes were redetermined. At this time also a second injection of sodium thiocyanate was given. At exactly one hour following the end of the infusion (two hours after the beginning of the infusion) blood was drawn for determination of the post-injection thiocyanate space and the total body water, the heavy water injected for the measurement of the latter having been allowed two hours to equilibrate. The fact that one hour was allowed for equilibration of the sodium thiocyanate, instead of the more commonly employed forty-five minutes, resulted in an apparently larger extracellular fluid volume than would otherwise have been the case; however, the same technique was used throughout, permitting valid comparison between pre-infusion and post-infusion data. One measurement only of total body water was performed.

In 10 patients the thiocyanate space was measured for a third time, three hours after the end of the infusion. As a rule, the post-infusion measurements of plasma electrolyte concentrations were made promptly at the end of the infusion and were not repeated. Thus, the changes in plasma electrolytes following infusion were measured one hour prior to the first post-injection measurement of the thiocyanate space.

biologic effects of such fluctuations? The answers to these and a host of related problems await further data regarding the volume and composition of the intracellular fluid in health and in disease.

In a series of scholarly articles, Elkinton and his associates[1-3] have reviewed certain evidence concerning the transfer of water and electrolytes across the cell membrane in heart failure—exchanges which have broadly applicable physiologic implications. As an example, whereas it has commonly been assumed that the important abnormality in fluid distribution in cardiac decompensation is the retention of salt and water in the extracellular space, these workers conclude from their studies that the intracellular phase shares in the abnormal fluid deposits. The intracellular derangements consist of potassium depletion, an increased osmolarity of the solutes present, and *overhydration of the cells*

Cellular Overhydration and Water Intoxication. In 1922 Rowntree and his associates[4] described a syndrome which they termed water intoxication. The clinical findings included convulsions which occurred when a patient with diabetes insipidus continued to drink large quantities of water after polyuria had been controlled with Pitressin. It was found that this condition could be reproduced consistently in animals, and that the volume of water ingested was of less importance than the concentration of salt in the extracellular fluid[5] It was virtually impossible to produce the syndrome if the administered fluid contained enough salt In 1948 Schroeder[6] called attention to the group of findings which may appear in patients in heart failure who are placed on a rigid low salt diet, allowed unlimited quantities of water, and perhaps given mercurial diuretics. He concluded that the low extracellular salt concentration which developed in these patients permitted overhydration of the cells (water intoxication) with resulting renal dysfunction and central nervous system manifestations The intravenous administration of hypertonic sodium chloride solution was markedly beneficial in effecting improvement in symptoms and renal function.

That water intoxication may occur in surgical patients has long been recognized. In a recent report, Zimmermann and Wangensteen[7] review the experience of others and cite 17 patients in whom they observed signs and symptoms of water intoxication in the early postoperative period Most of these individuals exhibited a sharply positive water balance. The administration of hypertonic sodium chloride solution produced a marked clinical improvement These investigators concluded that the alterations in endocrine activity which follow major operations, especially those involving the adrenal cortex and the posterior pituitary, render the postoperative patient especially susceptible to water intoxication because of a temporary inability to excrete a water load normally.

From the foregoing considerations, it is evident that problems having to do with variations in the degree of cellular hydration are important in surgical practice.

The studies herein reported were prompted by the remarkable improvement which occurred in a patient with duodenal obstruction who was given 500 cc. of 5 per cent sodium chloride solution intravenously. Contrary to preinjection estimates, the previously lowered serum sodium and chloride levels were only moderately elevated following this infusion but the pulse, previously rapid and weak, became slow and full. The patient appeared and

felt much improved. Nevertheless, it did not appear reasonable to assume that the marked improvement in the signs and symptoms of extracellular water and *salt* depletion was due entirely to the moderate elevation of the depressed serum sodium and chloride levels, in the absence of significant alterations in extracellular fluid and plasma volumes. To examine this question in a quantitative manner, a liter of 3 per cent sodium chloride solution was administered intravenously to 30 patients, without untoward symptoms. In 17 patients the infusion was completed in precisely one hour, and the results in these subjects form the basis of the present report except where otherwise indicated.

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METHODS

Adults in good general health awaiting elective operations were selected without regard to degrees of obesity. Control measurements of plasma carbon dioxide combining power, chloride, sodium and potassium were made. The plasma volume (Evans blue dye), red cell mass (plasma volume and venous hematocrit), extracellular fluid (NaSCN), and total body water (D_2O) were determined. The intracellular fluid volume was obtained by subtracting the thiocyanate space from the measured total body water. Red cell sodium and potassium were determined with the Beckman Model B flame spectrophotometer, as were the plasma and urinary concentrations of these ions

After these preliminary measurements, one liter of 3 per cent sodium chloride solution was infused intravenously in one hour. Promptly at the end of this time blood was drawn for repeat plasma electrolyte measurements, and the plasma and total blood volumes were redetermined. At this time also a second injection of sodium thiocyanate was given At exactly one hour following the end of the infusion (two hours after the beginning of the infusion) blood was drawn for determination of the post-injection thiocyanate space and the total body water, the heavy water injected for the measurement of the latter having been allowed two hours to equilibrate. The fact that one hour was allowed for equilibration of the sodium thiocyanate, instead of the more commonly employed forty-five minutes, resulted in an apparently larger extracellular fluid volume than would otherwise have been the case; however, the same technique was used throughout, permitting valid comparison between pre-infusion and post-infusion data. One measurement only of total body water was performed.

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RESULTS

Changes in Body Fluid Compartments. *Extracellular and Intracellular Spaces* It may be seen in Table 1 and in Figure 1 that one hour following the end of the infusion, which had itself lasted one hour, the "extracellular fluid" (thiocyanate space) had increased from an average of fifteen liters to an average of twenty liters. There was little further change three hours later (Table 1). Thus, it would appear that four liters of intracellular fluid had shifted to the extracellular compartment, assuming that the fifth liter by which the extracellular volume was increased represented the one infused. The average percentage of body weight represented by total body water in these patients was not particularly low (average 56 per cent). Therefore, the relatively high percentage of body water represented by the

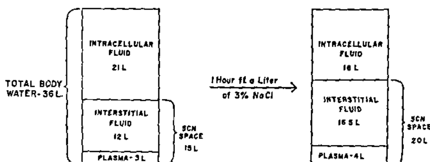


Fig 1 The thiocyanate space was increased from an average of approximately 15 liters to an average of approximately 20 liters by the saline infusion This represented a shift of four liters of water from the intracellular to the extracellular fluid compartment, assuming that the fifth liter by which the "extracellular fluid" (thiocyanate space) was increased represented the one infused The plasma volume (in the 7 patients so studied) increased from 3 liters to 4 liters The hematocrit decreased an average of 10 per cent.

Table 1. Results Following Intravenous Infusion of One Liter of 3 Per Cent Sodium Chloride Solution in 18 Patients

VARIABLE	PER CENT CHANGE	NO OF CASES
Thiocyanate space		
at 1 hour	+33	17
at 3 hours	+30	10
Plasma volume	+30	7
Intracellular fluid	−22	17
Hematocrit	−10	10
Red cell mass	0	5
Plasma potassium	− 4	12
Plasma chloride	+10	18
Plasma sodium	0	16
Plasma CO_2-combining power	− 6	16
Red cell potassium	0	8
Red cell sodium	0	8

Urinary excretion data for duration of experiment (8 cases)· volume (H_2O), 210 cc; sodium, 43 mEq; potassium, 11 mEq.

thiocyanate space could have been due in part to the fact that some of the individuals were obese, for the thiocyanate space of obese individuals is likely to represent a greater percentage of body water than in lean individuals.[9] Moreover, as noted, the thiocyanate was allowed to remain one hour before the blood sample was drawn for analysis, instead of the more commonly employed interval of forty-five minutes; the longer this substance is allowed to remain in the body, the greater is the apparent extracellular fluid volume. Nevertheless, again, the same technique was used both before and after the infusion.

Plasma and Blood Volume. The plasma volume increased from an average of three liters to almost four liters in the 7 subjects so studied. This represented an increase of approximately 30 per cent. The total blood volume increased an average of 17 per cent; the red cell mass was unchanged.

Changes in Plasma Electrolyte and Other Values. In Table 1 are shown the changes which occurred in the concentrations of plasma sodium, chloride, potassium, and carbon dioxide combining power. The data for the hematocrit and the red cell sodium and red cell potassium measurements are also given, as well as certain measurements of urine volume and the urinary excretion of sodium and potassium during the experiment.

The plasma carbon dioxide combining power decreased an average of 6 per cent in the 16 cases so studied. This was a reflection of the fact that the infusion of sodium chloride solution has a net acidifying effect, due to the "physiologic excess" of chloride ions. That is, the normal plasma sodium level of 142 mEq. per liter and the plasma chloride level of 103 mEq. per liter do not correspond to the 145 mEq. of sodium and the 145 mEq. of chloride contained in 0.85 per cent sodium chloride solution, the so-called "physiologic saline."

There was no significant change in the sodium level or in the potassium level, but there was a definite increase in the plasma chloride concentration. The hematocrit declined an average of 10 per cent in the 10 patients so studied. No change occurred in the red cell sodium and in the red cell potassium concentrations. There was an insignificant loss of water, sodium, and potassium in the urine during the experiment.

DISCUSSION

To return to the initial objective of this series of experiments, our purpose was to determine whether or not the clinical improvement in the patient with duodenal obstruction that we had treated could have been due to an increase in the extracellular fluid volume at the expense of the intracellular compartment. The data indicate that it was. The evidence which favors the increase in extracellular volume at the expense of intracellular volume does not rest entirely upon the apparent increase in thiocyanate space, a measurement which is open to some criticism. There was, in addition, a 30 per cent increase in the plasma volume (from three to almost four liters), despite the fact that a crystalloid and not a colloid solution had been infused. Moreover, while the total blood volume was increased, the increment was essentially due to an increase in the plasma volume, since the red cell mass, as calculated from the plasma volume and the hemotocrit, did not change appreciably. Furthermore, the hemotocrit diminished an average of 10 per cent. Finally, the chloride concentration increased an average of only 10 per cent in 18 patients so studied.

CONCLUSIONS

The present studies would appear to support the conclusion that the intravenous administration of hypertonic sodium chloride solution is effective in the management of convulsions due to water intoxication because it withdraws water from the cells. Similarly, hypertonic saline (when indicated to rectify hypo-osmolarity of the extracellular fluid) may alleviate certain of the signs and symptoms of extracellular water and salt depletion by augmenting the volume of this space at the expense of the intracellular compartment.

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STUDIES WITH VITAMIN B_{12}-Co^{60} *

LLOYD D. MACLEAN

The investigations of Castle and co-workers during the past quarter century strongly support the view that pernicious anemia is the result of a gastric defect manifested by an inadequate production of a still unidentified substance, "intrinsic factor," which accomplishes the absorption from the gastro-intestinal tract of "extrinsic factor," now definitely identified as vitamin B_{12}.[1] If intrinsic factor is produced solely by the stomach, one would expect the invariable development of pernicious anemia following total gastrectomy. This is, in fact, a rarely reported complication The infrequent occurrence of megaloblastic anemia following total gastrectomy suggests the possibility of an extragastric source of intrinsic factor in the human, a situation known to obtain for the hog.[2] Preparations of hog ileum, colon, jejunum

* From the Department of Surgery, University of Minnesota Medical School, Minneapolis *This study was supported by U S P H S grant No A382C*

and duodenum have been reported to contain intrinsic factor activity.[3] Human saliva is believed by some to have intrinsic factor activity.[4]

Pernicious anemia has been successfully treated with Aureomycin,[5] which revives the theory, prominent at the turn of the century, that intestinal sepsis plays a role in the pathogenesis of pernicious anemia.

In light of this evidence the infrequent occurrence of pernicious anemia following total gastrectomy may be the result of one or a combination of the following: (1) an extragastric source of intrinsic factor exists in the human; (2) pernicious anemia is a more complex deficit than a simple intrinsic factor lack; (3) patients upon whom total gastrectomy is performed usually have advanced carcinoma and the survival period after operation may not be sufficiently long to allow the development of pernicious anemia; (4) a complete removal of all gastric mucosa may not be accomplished in many gastrectomies believed to be total; (5) hematopoietically active substances such as folic acid may be ingested unknowingly by the patient in compound vitamin pills or parenteral liver extract, or vitamin B_{12} may be given for prophylactic purposes.

Megaloblastic anemia actually does occur occasionally after gastrectomy and the possibility exists that it is a nutritional anemia, either a folic acid deficiency or an exogenous vitamin B_{12} deficit.

Since 1948 quantitative microbiological techniques for vitamin B_{12} assay have been available, making feasible intake-output (balance) studies. Many of the above mentioned theories could be delineated if one could administer vitamin B_{12} orally and calculate the percentage excretion in the stool and urine. The production of vitamin B_{12} by bacteria in the colon, however, makes such an investigation of doubtful value since the origin of the fecally excreted vitamin B_{12} is unknown.

Vitamin B_{12} made radioactive by the substitution of Co^{60} for the normally present cobalt atom in the vitamin B_{12} molecule became available for clinical trial in 1950,[6] and the above suggested investigation was performed by Heinle et al.[7] on normal and pernicious anemia patients. These workers found that while normal individuals excreted less that 25 per cent of an orally administered dose of 0.5 micrograms of vitamin B_{12}-Co^{60} in the stools, patients with pernicious anemia fecally excreted over 75 per cent of this dose. The addition of a source of intrinsic factor, if given to pernicious anemia patients with the oral radioactive vitamin B_{12}, reduced their fecal excretion to within normal limits.

Our investigation is concerned with the absorption of orally administered vitamin B_{12}-Co^{60} as an assay of intrinsic factor in the following groups: normal individuals, achlorhydric patients, pernicious anemia patients, and patients with total gastrectomy and with proximal subtotal gastrectomy with esophagoantrostomy.

SECTION I

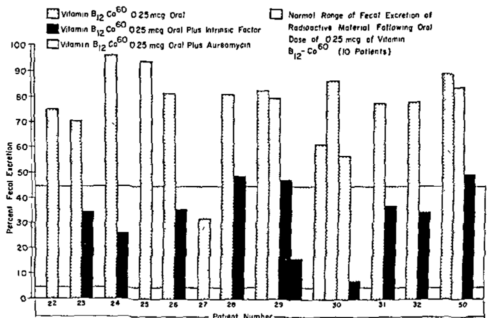
Methods

All investigated patients were admitted to the hospital for 7 to 10 days and given an oral dose of vitamin B_{12}-C^{60}* containing 0.25 micrograms of vitamin B_{12} and 0.022 microcuries of cobalt[60].† All stools were collected on

* Obtained from Dr. Charles Rosenblum, Merck and Co., Rahway, N. J.
† United States Atomic Energy Commission authorization No. 25324.

wax paper, quantitatively placed in 60 ml. plastic counting tubes and the output of radioactivity was determined. The counting device used was a Texas Cup Geiger counter.* The total amount of radioactivity in the administered dose was determined in counts per minute, using the same counter. Stool collections were continued until no significant amount of radioactivity could be detected. Pilot experiments revealed no significant urinary excretion of radioactive material under these conditions.

During the last 6 month period a more sensitive well-scintillation counter has been used. The stools are collected in the same manner, dried for 24 hours in an evaporating dish in an oven at 110°C. and ashed in a muffle



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furnace at 600°C for 15 hours. The ash is counted in 5 ml. patent lip vials under constant geometry. Recovery experiments have shown a loss of less than 5 per cent using this method when a known amount of vitamin B_{12}-Co^{60} is added to a normal stool. The containers used for administering the dose were counted before and after administration in each case and the true dose ingested was determined.

Results

Fecal Excretion of Vitamin B_{12}-Co^{60} in Normal Individuals. The patients used for controls in this investigation had a normal gastro-intestinal tract, no previous abdominal operations, and normal hemoglobin. The fecal excretion of radioactivity in all cases was less than 44 6 per cent (Fig. 1).

Fecal Excretion of Radioactive Material in Pernicious Anemia Patients.

* Kindly donated to the University of Minnesota by The Texas Company, Houston, Texas.

In contrast to the normal group, pernicious anemia patients excreted over 65.1 per cent of the oral dose of vitamin B_{12}-Co^{60} whether the anemia was in relapse or remission. The fecal excretion in 2 cases was reduced to normal (12.4 and 31.2 per cent) by the addition of intrinsic factor.*

Fecal Excretion of Orally Administered Vitamin B_{12}-Co^{60} in Patients with Microscopically Proved Total Gastrectomy. Twelve patients have been studied by the method outlined and 11 excreted amounts of radioactive material in the feces in quantities completely comparable to those excreted by pernicious anemia patients. Table 1 illustrates the clinical and laboratory data [illegible] Four of the 12 patients [illegible] nediately preceding the [illegible] it pernicious anemia in these patients prior to operation. The type of reconstruction of the alimentary tract following total gastrectomy, whether by Roux-Y, esophagoduodenostomy or loop esophagojejunostomy, did not alter absorption in the cases studied.

A marked decrease in the fecal excretion of radioactive material occurred when a source of intrinsic factor, either as normal human gastric juice or as intrinsic factor concentrate, was given with the vitamin B_{12}-Co^{60} (Fig. 1).

Aureomycin in doses of 250 mg. every 6 hours, started 24 to 48 hours prior to oral vitamin B_{12}-Co^{60}, did not influence the fecal excretion of radioactive material (Fig. 1).

Fecal Excretion of Radioactive Material in Achlorhydric Individuals. Six patients with histamine-fast achlorhydria were investigated as outlined above. The fecal excretion of radioactive material was normal in all cases ranging from 5.6 to 28.8 per cent. The patients ranged in age from 47 to 68 years; none had gastric polyps.

Fecal Excretion of Radioactive Material in Patients with Proximal Subtotal Gastrectomy and Esophagoantrostomy. Three patients have been studied in this group In 2 the operation was performed for gastric carcinoma, in 1 for cardiospasm and esophagitis. The latter patient had 35° of free acid on gastric analysis preceding the operation. All resected specimens were examined microscopically and revealed esophageal mucosa at the proximal line of resection and antral mucosa at the distal line. The fecal excretion of radioactive material was markedly above normal in all 3 patients, whereas this investigation performed on one of the patients prior to operation revealed a normal excretion. In another one of the 3 patients fecal excretion of radioactivity was decreased to normal by the simultaneous oral administration of intrinsic factor concentrate with the vitamin B_{12}-Co^{60} (Table 2).

SECTION II

A urine modification of the above test has been described by Schilling,[8] based on the fact that 38 to 100 per cent of an intramuscular dose of 1000 micrograms of vitamin B_{12} is excreted in the urine within 24 hours.[9] An oral dose of vitamin B_{12}-Co^{60} is administered. In the individual producing intrinsic factor an appreciable quantity is absorbed depending upon the size of the dose Within 2 hours of the oral dose of radioactive vitamin B_{12}, the "flushing" dose of 1000 micrograms of non-radioactive vitamin B_{12} is given

* Intrinsic factor was administered as 100 ml of normal human gastric juice or 220 mg of intrinsic factor concentrate, kindly supplied by Dr. K. W. Wantland of Eli Lilly Co, Indianapolis, Indiana.

Table 1. Clinical and Laboratory Data of Interest on Total Gastrectomy Patients

PATIENT	DIAGNOSIS	DATE OF OPERATION	FREE HCL BEFORE OPERATION	BONE MARROW BIOPSY	RECONSTRUCTION OF ALIMENTARY CANAL	PER CENT OF FECAL EXCRETION OF ORAL VIT B_{12}-Co^{60}
22	Adenocarcinoma of stomach	6-2-52	39°	—	Roux-Y	74 4
23	"	2-2-53	26°	—	"	70 4
24	"	6-4-51	0°	Megaloblastic on 9-4-54	"	97 0
25	"	3-21-52	0°	—	"	94 0
26	Benign gastric ulcer	1-4-52	36°	—	"	81 7
27	Adenocarcinoma	9-12-52	0°	—	"	31 5
28	"	11-13-51	0°	Normal on 4-10-54	"	81 2
29*	"	7-3-53	0°	Normal on 9-5-54	Esophagoduodenostomy	82 6
30	"	3-1-49	0°	Megaloblastic on 4-20-54	Esophagojejunostomy	61 0 86 9 56.7
31	"	5-8-53	0°	—	Roux-Y	77.4
32	Malignant lymphoblastoma	11-25-53	0°	Normal on 10-2-54	Esophagoduodenostomy	78 4
50	Adenocarcinoma of stomach	10-23-50	31°	Megaloblastic on 9-6-54	Esophagojejunostomy	89 5

*This patient received prophylactic therapy, vitamin B_{12} since gastrectomy.

intramuscularly. Thirty-eight to 100 per cent of both the absorbed and intramuscular vitamin appears in the urine during the subsequent 24 hours in normal individuals. In pernicious anemia patients insignificant absorption from the gastro-intestinal tract occurs; therefore, greatly decreased amounts of radioactivity are detectable in the urine.

In preliminary examinations we have found extremely small amounts of radioactivity in the urine of total gastrectomy patients under the conditions of this test, the amount being elevated to near normal by the addition of a source of intrinsic factor with the vitamin B_{12}-Co^{60}. There is a simultaneous decrease in fecal excretion with the increase in urinary excretion of radioactive material which is dependent upon intrinsic factor.

The possible usefulness of this simple urine test to differentiate achylia gastrica from achlorhydria has been investigated particularly with the

*Table 2. Fecal Excretion of 0.25 Micrograms of Vitamin B_{12}-Co^{60} in Patients with Proximal Subtotal Gastrectomy and Esophagoantrostomy with and without Intrinsic Factor Concentrate**

PATIENT	DIAGNOSIS	FREE HCL BEFORE OPERATION	TEST DOSE OF VITAMIN B_{12}-Co^{60} IN MICROGRAMS	TEST DOSE OF INTRINSIC FACTOR	PER CENT EXCRETION
33	Cardiospasm	35°	0 25		84.4
			0 25	220 mg of intrinsic factor concentrate	23.0
34	Adenocarcinoma of stomach	0°	0 25†		21 4
			0 25		73 9
35	Adenocarcinoma of stomach	0°	0 25		99.5

*All patients were operated upon within the last 12 month period
None have received prophylactic therapy and none have developed pernicious anemia.
†Test done immediately prior to operation, reveals a normal excretion

thought of identification of gastric cancer precursors. Gastric cancer occurs in 5 to 6 per cent of pernicious anemia patients in contrast to 0.9 per cent of achlorhydric individuals and 0.1 per cent of the general population.

Method

The test is performed only on histamine-fast achlorhydria patients. It is begun following breakfast at 8 00 A.M. and immediately after urination. An oral dose of 1.0 microgram of vitamin B_{12}-Co^{60} with a specific activity of 760 millicuries per milligram is given orally followed immediately by a "flushing" dose of 1000 micrograms of non-radioactive vitamin B_{12} intramuscularly. All urine is collected for 24 hours Five 5 ml. aliquots are taken and [illegible] the form of vitamin B_{12}-Co^{60} and not a degradation product.[10]

To date, 5 normal individuals, 30 achlorhydric patients without gastric

polyps, 5 patients with gastric polyps and 5 pernicious anemia patients have been studied. The results are summarized in Table 3.

The simplicity of this test utilized to differentiate true achylia from achlorhydria recommends it for cancer detection purposes or for convenience in early and precise diagnosis of pernicious anemia. The possible interference of impaired renal function can be eliminated by noting the

Table 3 Percentage Urinary Excretion of 1 Microgram of Orally Administered Vitamin B_{12}-Co^{60}

GROUPS INVESTIGATED	NO OF PATIENTS	PER CENT URINARY EXCRETION
1. Normal	5	19–31
2. Pernicious anemia	5	<1
3 Achlorhydria without gastric polyps	30	12–32
4 Gastric polyps	5	10-17
5 Achylia—differentiated from achlorhydria by means of test	1*	<1

* *This patient excretes no uropepsin Blood smear, hemoglobin and bone marrow are* normal Urinary excretion of vitamin B_{12}-Co^{60} became 15 per cent on addition of 220 mg purified intrinsic factor given with dose of vitamin B_{12}-Co^{60}

normal urinary excretion of radioactive material when intrinsic factor is given orally with the vitamin B_{12}-Co^{60}.

Discussion

Utilizing the percentage fecal excretion of orally administered vitamin B_{12}-Co^{60} as an assay of intrinsic factor in the gastro-intestinal tract has revealed that patients with microscopically proved total gastrectomy fecally excrete over 65 per cent of orally administered vitamin B_{12}-Co^{60} in comparison to normals with less than 44 per cent. The excretion can be greatly reduced by the addition of intrinsic factor, a situation comparable to that found in pernicious anemia patients. The abnormally elevated fecal excretion of radioactive material under these conditions prevailed whether the patient produced free acid prior to the operation or not, and in the absence or presence of a megaloblastic bone marrow. Three of the patients investigated had a megaloblastic bone marrow. All of these with megaloblastic anemia had undergone total gastrectomy over 3 years prior to its onset. Ninety-nine per cent of pernicious anemia patients in whom therapy has been withdrawn will go into relapse before 3 years, 69 per cent within the first year [11] The time required to develop pernicious anemia initially is not known, however, achlorhydria has been noted as long as 25 years prior to the onset of anemia

It is felt that true pernicious anemia is inevitable following true total gastrectomy or proximal subtotal gastrectomy with esophagoantrostomy if the patients survive over three years and
in the form of parenteral liver extract or
ports the view, originally stated by Fo:
fundus of the stomach are the only clinically significant sites of intrinsic factor production in the human and that pernicious anemia is the manifestation of this deficit. Halsted and coworkers[14] have recently reported comparable results in patients after total gastrectomy.

Aureomycin did not influence the absorption of vitamin B_{12}-Co^{60}, which

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A QUANTITATIVE STUDY OF THE ANATOMIC DISTRIBUTION OF INTRAVENOUS LIPID EMULSIONS*

J. E. BEVILACQUA, G. L. KRAUSE, JR., R. E. BOTTI,
AND OTTO ROSENTHAL

Up to the present time, work with fat emulsions for intravenous nutrition has centered chiefly on the practical aspects of manufacture, safety and convenience of administration and proof of utilization. Although some work has been reported on the sites of deposition and utilization of the emulsions,[1-3] a direct quantitative estimation under controlled conditions in the experimental animal has yet to be reported.

In this study the deposition of an intravenous fat emulsion in five sites—lung, liver, spleen, ileum and skeletal muscle—was examined in ten normal dogs.

METHODS

Under pentobarbital anesthesia an intravenous fat emulsion (Merck & Co.) containing 10 grams of sesame oil stabilized with lecithin in 100 ml. of 5 per cent glucose solution was infused into a leg vein at a rate of 2.0 to 4.0 ml. per minute. The organs to be studied were biopsied immediately

* From the Harrison Department of Surgical Research, Schools of Medicine, University of Pennsylvania, Philadelphia.

before and after the infusion. Each biopsy sample was divided into six portions. Two pieces served for duplicate assays of the total lipid content while the remaining four were used for determination of the water content and histologic examination.

Lipids were extracted from the minced tissue by hot alcohol-ether solutions. After evaporation of the solvent the residue was taken up in petroleum ether and the quantity of the material extracted determined gravimetrically. This value was taken as the fat content of the sample.

To evaluate the significance of changes in the fat content of the tissues the standard error of observation[4] was computed from the mean variance of the duplicate determinations in all dogs. From this the standard error of the difference between the pairs of pre- and postinfusion samples was calculated. This figure is listed in Table 1 for each organ. In two dogs which

Table 1. Lipid Extraction Results

EXP. NO.	LIPID GM./KG.	LIPID INCREASE IN GM /100 GM. TISSUE LUNG	LIVER	SPLEEN
1	0	0.20	0.21	−0.12
2	1	0.18	1.20	0.93
3	1	−0.15	1.67	0.57
4	1		0.85	1.83
5	2	−0.50	1.78	4.07
6	2	0.10	2.69	5.90
7	4		1.60	10.15
8*	0		0 50	−0.25
9*	1	0.61	0.00	1.90
10*	1	0 48	1.30	0.07
Standard error of difference between two means†		0.399	0.437	0.305

* Animals received heparin.
† Computed from the standard error of measurement.[4]

received only a 5 per cent glucose solution instead of the fat emulsion the differences between the pre- and postinfusion samples were within the limit of sampling errors.

The lipid extractions from the ileum and skeletal muscle gave variable results, owing probably to sampling difficulties, and will not be reported. Microscopic sections were stained for free fat with Scharlach R. Organ weights were determined at sacrifice of the animal immediately after the procedure.

In addition, blood lipid levels were followed in several different vessels by a macro-modification of the turbidity method of Geyer, Mann and Stare.[5]

RESULTS

Chemical Fat Content. Since no significant changes in the water content of the tissues were found following administration of the fat emulsion, the composition of the pre- and postinfusion samples could be directly compared on a wet weight basis.

The changes in total lipid concentration per 100 grams of tissue are recorded in Table 1. When the animal received 1.0 gram of fat per kilogram of body weight, the fat extractable from the liver and spleen increased by approximately 30 per cent. At an infusion level of 2.0 grams per kilogram

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* From the Harrison Department of Surgical Research, Schools of Medicine, University of Pennsylvania, Philadelphia.

demonstrable microscopically at 1.0 gram per kilogram—the smallest infusion studied. No fat accumulation was observed in the lung, ileum or *skeletal muscle*.

The fat droplets were contained within the Kupffer cells of the liver and were unevenly distributed throughout the sections. In regions of deposition the fat appeared to be more heavily concentrated in a band occupying the mid-third of the lobule in cross section. The appearance of the liver after the infusion of 2.0 grams of fat per kilogram is illustrated in Figure 1. Fat particles appear as dark dots and can be seen clustered within the cytoplasm of several of the Kupffer cells in the field.

Figure 2 shows the condition of the spleen after the administration of 2.0 grams of fat per kilogram. The fat is clustered around the smaller

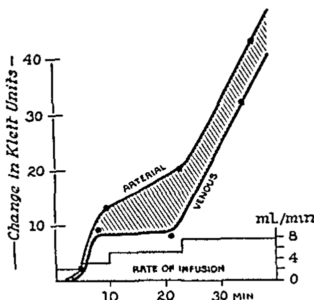


Fig 3 Illustrating one series of blood lipid levels in the splenic artery and vein during the intravenous administration of a fat emulsion.

arterioles, chiefly of the sheathed variety. It also lies in streaks and clusters through the sinuses. Some of the sinusoidal fat is within phagocytes. No fat was seen in the white pulp or around the central arterioles.

Blood Lipid Levels. While following the blood lipid levels in several vessels an arteriovenous difference across the splenic circulatory bed during the infusion was demonstrated The time course of an illustrative procedure is presented in Figure 3. As the rate of infusion is increased, both arterial and venous levels rise, but the arterial concentration goes up faster than the venous level. The arteriovenous difference varies with the rate of infusion and the amount of fat that has been given. This latter factor presumably reflects the state of saturation of the organ.

DISCUSSION

From these observations it would seem that one-third to one-half of the fat given by an intravenous emulsion localizes immediately in the liver. Although the spleen takes up a large amount of the fat relative to its mass,

...cent in the liver and more than 60 per cent in ... amounted to 400 per cent ... the splenic increment ... all and not statistically significant.

Because of its clearing effect on lipemic plasma[3] heparin was given in small doses to three animals--one a control in which only 5 per cent glucose was infused. As can be seen in Table 1, the results were inconsistent.

In Table 2 the increases in total lipid content of the organs are recorded as percentages of the quantity of fat administered. Immediately after the infusion about 45 per cent of the fat was found in the liver at both the 1.0 and 2.0 grams per kilogram infusion levels. Deposits in the spleen amounted to 3 per cent of the amount given when 1.0 gram of fat per kilogram was administered, and to 8 per cent when 2.0 grams per kilogram was injected.

Table 2. Percentage of Total Infused Fat Recovered in Liver and Spleen

EXP. NO	LIPID GM./KG.	LIVER	SPLEEN
2	1	38	3.2
3	1	53	2.8
5	2	24	8.5
6	2	48	7.5

Histologic Fat Distribution. In both liver and spleen fat deposition was



Fig 1 Fig 2.

Fig. 1. Section of liver (600 ×) after the infusion of 2 grams of fat per kilogram of body weight. Kupffer cells are prominent and many are filled with minute fat droplets, represented in the picture by dark particles

Fig 2 Section of spleen (230 ×) after the infusion of 2 grams of fat per kilogram of body weight. Dark particles represent minute fat droplets. They are clustered about smaller arterioles and also lie in the sinuses in streaks.

demonstrable microscopically at 1.0 gram per kilogram—the smallest infusion studied. No fat accumulation was observed in the lung, ileum or skeletal muscle.

The fat droplets were contained within the Kupffer cells of the liver and were unevenly distributed throughout the sections. In regions of deposition the fat appeared to be more heavily concentrated in a band occupying the mid-third of the lobule in cross section. The appearance of the liver after the infusion of 2.0 grams of fat per kilogram is illustrated in Figure 1. Fat particles appear as dark dots and can be seen clustered within the cytoplasm of several of the Kupffer cells in the field.

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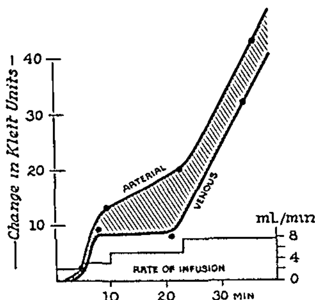


Fig. 3. Illustrating one series of blood lipid levels in the splenic artery and vein during the intravenous administration of a fat emulsion

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DISCUSSION

From these observations it would seem that one-third to one-half of the fat given by an intravenous emulsion localizes immediately in the liver. Although the spleen takes up a large amount of the fat relative to its mass,

the actual proportion of the total quantity given is small when smaller amounts are given.

These results conform to the pattern established by Waddell et al.,[2] who found that the absence of the liver greatly decreased the rate of clearance of the fat from the blood stream. They also are similar to the findings of Grossman and Strub [3] These authors report finding in the rat a relatively high splenic uptake, which, however, is small in total quantity, and a deposition in the liver which in total quantity is large. In their animals the administration of heparin apparently resulted in a diminished accumulation in the spleen.

The histologic findings are substantially in accord with those of Murray and Freeman,[1] who demonstrated the appearance of fat droplets in microscopic sections of liver and spleen after the administration of 0 8 grams per kilogram. However, these authors reported on the lungs of rats only, and in that animal, after administration of their emulsion, fat was observed in the lung.

The significance of the anatomic distribution of infused fat is as yet unknown. Obviously the mere fact of deposition in an area does not prove that utilization takes place in that site. Murray and Freeman have pointed out the difference between fatty chyle slowly formed by the intestine, which goes to hepatic parenchymal cells, and artificial fat emulsions, which are deposited in reticulo-endothelial cells. Thus deposition patterns are the result of the primary processing of the material. For example, the reticulo-endothelial cells may serve to break down macromolecular complexes and form smaller lipoproteins or other compounds—much as do the cells of the intestinal mucosa—so that the fat can be handled in the body's metabolic pathways. Perhaps the heparin activation of plasma enzymes is the reason for the reported decrease in splenic deposition when that compound is given.

Moreover, only half of the fat has been traced The portion whose immediate fate is known seems to be treated as a foreign material. This leads to the speculation that perhaps the emulsion is heterogeneous, with the untraced half *immediately available to the cells for utilization or storage in* depots Certainly a small amount of the fat does enter metabolic paths at once.[5-7]

Heparin apparently does affect the handling and deposition of fat given by intravenous emulsion, but the exact nature of its effect and its mechanism of action are unknown.

Clearly the emulsion tested in this study does not produce fat embolism in the lungs of dogs.

SUMMARY

Biopsies of lung, liver and spleen taken from normal dogs before and immediately after the intravenous administration of a fat emulsion were compared by extraction of total lipids and by microscopic section.

An increase in lipid content was demonstrated in liver and spleen, and fat droplets appeared in the sections The deposition in these two organs accounted for slightly less than one-half of the fat infused. The reticulo-endothelial phagocytes seem to be the components of the liver and spleen that take up the fat.

No increase was found in lung lipid, nor were fat droplets seen in the microscopic sections of that organ.

followed each dialysis, but his course was progressively downhill. He died on the nineteenth post-injury day.

Figure 1 illustrates the plasma NPN fluctuations in this patient. With the exception of the amino acids, all the NPN components showed consistent

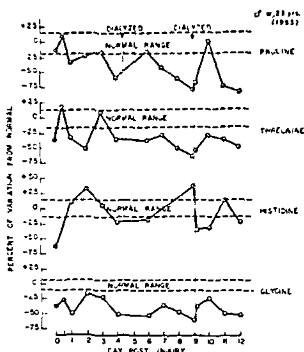


Fig 2. Plasma proline, threonine, histidine and glycine in a patient with severe battle wounds and renal failure

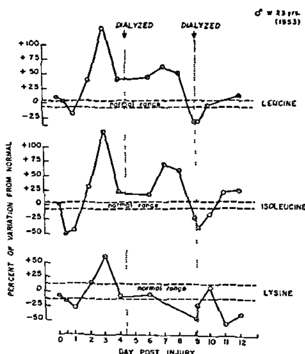


Fig. 3. Plasma leucine, isoleucine and lysine in a patient with severe battle wounds and renal failure.

3 1 mg. per cent There is considerable variation in concentration among the individual amino acids, but each amino acid is present in about the same relative concentration among normals. The plasma amino acids are in active metabolic exchange with tissue amino acids, tissue and plasma proteins, and body carbohydrate and fat. Since alterations in protein, fat, and carbohydrate metabolism follow injury, changes in amino acid metabolism can be anticipated.

We studied four fatally wounded soldiers. Shock and persistent renal failure were present in all The following case report typifies these patients:

A 26 year old American soldier was severely wounded by mortar shell fragments. His injuries included lacerations of the scalp, cerebral concussion, penetrating wounds of the abdomen, traumatic amputation of right thigh, and multiple soft tissue injuries. He was

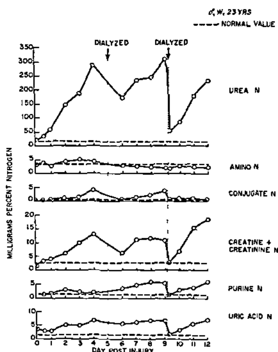


Fig 1 Plasma non-protein nitrogen in a patient with severe battle wounds and renal failure

in severe shock during the first three hours after injury, despite the administration of 2500 cc. blood, 350 cc 25 per cent albumin, and 1500 cc isotonic saline. His right leg

[illegible]

[illegible] consciousness During this time, he received another liter of blood His blood pressure had remained stable at about 130/80

[illegible] r atropine, Pentothal, and nitrous [illegible] and remained low throughout most [illegible] nal 3500 cc of blood Estimated [illegible] ee hours and consisted of exploratory laparotomy, drainage of the liver and kidney perforations, re-amputation of the thigh, and débridement of many soft tissue injuries

His postoperative course was complicated by profound and persistent renal failure, jaundice, pneumonia, wound infections, and considerable weight loss. Extracorporeal hemodialysis by a Kolff-type artificial kidney was necessitated on the fifth, ninth, and fifteenth days because of progressive hyperkalemia and uremia. Temporary improvement

Table 1. Effect of 6 Hour in Vivo Dialysis on Ultrafilterable Nitrogen Components of Plasma

PATIENT	PT. 1			PT. 4			PT. 5		
DAY POST INJURY	12			7			9		
	PRE-DIALYSIS	POST-DIALYSIS	% CHANGE	PRE-DIALYSIS	POST-DIALYSIS	% CHANGE	PRE-DIALYSIS	POST-DIALYSIS	% CHANGE
	MG. N/100 ML. PLASMA ULTRAFILTRATE								
NPN	392	118	−70	462	208	−55	383	76	−80
Urea N	336	97.5	−71	377	113	−70	311	54	−83
Creatine + creatinine N	9.7	5.5	−43	9.8	5.4	−45	10.8	3.0	−72
Uric acid N	6.7	2.5	−63	7.1	3.3	−54	6.9	1.6	−77
Purine N	3.8	0.9	−76	4.3	1.8	−55	5.2	1.1	−79
Amino conjugate N	3.1	0.8	−74	2.1	1.3	−40	4.0	0.3	−92
Amino N	2.7	2.8	+ 3	4.1	4.8	+17	2.0	1.9	− 5
	µM/100 ML. PLASMA ULTRAFILTRATE								
Aspartic acid	3.7	2.9		5.4	5.6		2.3	1.8	
Threonine	6.2	6.6		13.6	24.0		4.8	6.2	
Glutamic acid	8.5	10.4		18.0	14.1		11.8	12.5	
Proline	14.4	16.0		29.8	33.0		5.3	8.0	
Glycine	15.8	16.6		22.2	29.5		10.1	14.7	
Alanine	20.7	26.8		28.2	55.1		10.4	17.2	
Valine	17.0	19.2		22.9	27.3		14.6	9.2	
Methionine	3.5	2.2		6.6	7.5		Trace	Trace	
Isoleucine	6.8	5.2		11.7	7.4		5.8	4.5	
Leucine	9.6	12.3		17.7	19.1		8.0	8.0	
Tyrosine	4.0	5.1		8.6	11.2		1.7	1.7	
Phenylalanine	12.0	11.3		12.1	14.7		10.0	8.0	
Histidine	28.2	18.7		22.0	28.0		19.5	9.3	
Lysine	11.4	13.0		29.6	36.2		8.3	12.6	
Taurine	1.7	3.0		1.7	Trace		5.2	2.2	
Glutamine + serine + asparagine	29.1	26.1		40.0	30.3		22.0	18.4	

rises, falling only after dialysis. The plasma amino acids were only slightly elevated in the first 24 hours, despite severe shock and operation, and later slowly fell. Extracorporeal dialysis had no obvious effect on the amino nitrogen levels.

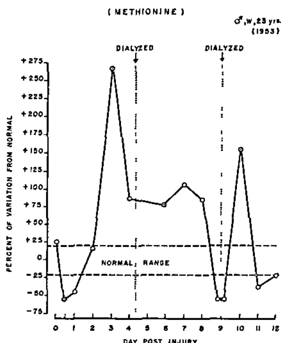


Fig 4 Plasma methionine in a patient with severe battle wounds and renal failure.

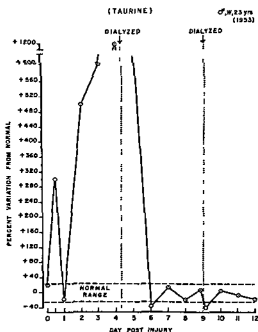


Fig. 5. Plasma taurine in a patient with severe battle wounds and renal failure.

The relative constancy of the total plasma amino nitrogen is not the result of unvarying concentrations of the individual amino acids. Rather, some of the amino acids rise, some fall, and some remain fairly constant. Because of the severe and persistent renal failure, urinary excretion of amino acids was negligible. The plasma concentration of glutamic acid was high early and fell later; proline, threonine, histidine, and glycine were low or normal (Fig. 2); leucine, isoleucine, lysine, valine, tyrosine, and alanine were elevated by the second or third post-wound day and fell later to normal or sub-normal levels (Fig. 3); phenylalanine, aspartic acid, and methionine also rose during the first week, but to a greater degree (Fig. 4). Taurine, which had risen to very high levels by the fourth post-injury day, fell sharply after the first dialysis and remained normal thereafter (Fig. 5). In contrast, the plasma concentrations of the other amino acids (Table 1) were little changed by the dialysis.

Early in our work, we noticed that one chromatographically distinct amino component could be hydrolyzed with hydrochloric acid to amino acids. In normals, this component is present in low concentrations, and glutamic acid and glycine comprise almost all of the conjugate. The plasma of the patients, however, contained the conjugate in greater quantity, and the component amino acids in greater variety. The plasma conjugate levels rose with the urea levels, though not proportionately. As the level of the plasma conjugate fraction rose, more and more different amino acids were found within the component, the quantitative relations of the amino acids showed an ever shifting pattern among the patients, and from day to day in the same patient. By the eleventh post-wound day, in this patient, 12 amino acids were present, with leucine and proline in addition to glycine and glutamic acid occupying quantitatively important positions (Table 2).

These observations suggested that the amino conjugate fraction is not homogeneous. Accordingly, we chromatographed the intact conjugate on paper. It was thereby separated into four components.

The conjugate, as a whole, acted as a metabolic end product with respect to extracorporeal dialysis. Its plasma concentration was reduced after dialysis in approximately the same ratio as urea. This is in marked contrast to the behavior of the plasma free amino acids, whose concentrations, in general, were little changed by dialysis.

In summary, we have found that total plasma amino nitrogen levels remained near normal in these patients, despite severe wounds, shock, operation, and renal failure. This relative constancy was not the result of normal individual amino acid levels, but was a fortuitous result of simultaneous concentration abnormalities. The amino acid levels were but slightly affected by extracorporeal dialysis, in contrast to the other NPN substances.

A heterogeneous amino conjugate was found and analyzed. Its composition varied from patient to patient, and in the same patient from day to day. This component rose, in general, with the plasma urea.

Table 2. *Amino Acid Composition of Plasma Ultrafilterable Amino Conjugate*

SUBJECT	PT 5					PT. 4				PT 3			PT 2	NORMALS	
DAY POST WOUND	½	1	2	11	12	½	2	7	8	3	8	11	10	A	B
	μM/100 ML PLASMA ULTRAFILTRATE														
Aspartic acid			4 0	3 4	2 5									1 7	
Threonine			1 8	3 2	2 0	3 6	2 3	7 9	2 3	15 8		0 8	13 3		
Serine			2 6	1 3	2 2	1 8	2 2	11 1	3 6	17 7		6 0	12 1		
Glutamic acid	6 4		10 8	13 0	20 8	6 6	2 1	9 5	6 7	18 5	8 0	4 7	41 2	8 7	5 5
Proline				10 1	0 8										
Glycine	18 1	5 3	18 0	26 0	10 0	15 1	8 8	29 5	11 6	37 5	23 5	74 4	145 0	6 6	8 8
Alanine	4 9	1 7		2 6	0 8	1 0		4 6					8 8		
Valine		2 0		2 5	1 5			9 5					41 2		
Leucine	4 8	4 0	10 0	11 5	10 0		21 7	45 6	31 0	5 4	45 2		57 2		
Tyrosine			4 8	6 0	5 2		5 3	23 4	10 6	4 2	19 1		36 4		
Phenylalanine				2 5											
Histidine			3 1	2 6	5 1	3 6		8 5	13 0	6 3	5 7		23 6		
Lysine						1 9									
Unknown			12 9		6 0		3 6		9 2						
Total	34 2	18 1	68 0	87 3	66 9	24 0	46 0	149 6	94 0	105 4	101 5	85.9	385.1	17 0	14 3
	MG N/100 ML PLASMA ULTRAFILTRATE														
Conjugate N	0 5	0 3	1 0	1 2	0 9	0 3	0 6	2.1	1 3	1.5	1 4	1 2	5.4	0.2	0 2
Urea N	32	57	115	177	234	36 4	132	377	178	84	122	42	364	20	17

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THE EFFECT OF ABDOMINAL OPERATIONS UPON THE SERUM AMYLASE AND SERUM LIPASE*

DWIGHT J. HOTCHKISS, JR., WILLIAM T. FITTS, JR., AND OTTO ROSENTHAL**

Acute postoperative pancreatitis is a complication of surgery which has received increasing attention during the past decade. Acute pancreatitis has been reported to occur not only after surgical procedures in the vicinity of the pancreas such as gastric resection, cholecystectomy and choledochostomy, and splenectomy, but also after subtotal thyroidectomy, appendectomy, cesarean section, and colon resection.

The seriousness and complexity of acute postoperative pancreatitis is excellently discussed in recent papers by Millbourn,[1] Warren,[2, 3] and Dunphy, Brooks, and Achroyd.[4] These authors point out that pancreatitis following abdominal surgery is not always due to trauma. Its cause in many instances remains obscure.

The importance of acute pancreatitis as a complication following surgery has induced several investigators to study the effects of operation upon the pancreatic enzyme, amylase. A significant increase in urinary amylase was observed by Usland[5] in 13 per cent, Bruusgaard[6] in 10 per cent, and Millbourn[1] in 9 per cent of patients following gastric resections involving no known pancreatic trauma Dunphy et al.,[4] however, found no significant elevations of serum amylase in a group of 50 patients undergoing all types of general abdominal surgery in which pancreatic injury and manipulation were excluded.

The present study is a further attempt to correlate postoperative alterations in serum amylase and lipase with various surgical procedures No similar study which has included both serum amylase and serum lipase determinations, and which has been done following operations outside of as well as within the abdomen, has been found in the literature.

METHODS

Forty-seven patients admitted to the Surgical Service of the Hospital of the University of Pennsylvania were divided into four groups, as follows:

Group I. Operations outside of the abdomen.

Group II. Abdominal operations remote from the pancreas.

Group III. Abdominal operations in close proximity to the pancreas, but not involving direct pancreatic trauma.

Group IV. An unclassified group including operations involving trauma to the pancreas, and several others.

Group I consists of 8 patients, 3 of whom underwent herniorrhaphy, 2, total adrenalectomy and Adson sympathectomy, 1, radical mastectomy; 1, radical neck dissection; and 1, mitral valvulotomy. In group II are 12 patients of whom 3 underwent exploratory laparotomy, 2, sigmoid colotomy and polypectomy; 3, large bowel resection, 2, total hysterectomy; 1, total

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** The guidance of Dr. John G Reinhold and the art work of Miss Elizabeth Marshall are appreciated.

cystectomy; and 1, intra-abdominal drainage of a pelvic abscess. Group III consists of 23 patients of whom 8 underwent cholecystectomy; 8, cholecystectomy and choledochostomy; 6, gastric resection; and 1, splenectomy. One patient underwent an exploratory laparotomy 10 days before a partial gastrectomy and is classified in group II for the first operation and in group III for the second. Group IV consists of 4 patients who could not be classified in any of the above three groups. They will be discussed separately.

In our study, blood samples were taken one day preoperatively, then postoperatively on days 1, 2, 4, and 6. All samples were drawn before breakfast and, on days of operation, before preoperative medication was given. Since these patients were cared for by many different surgeons, no attempt was made to control the type of anesthesia, of preoperative medication, and of postoperative drug therapy.

Somogyi's[7] method for estimation of serum amylase was employed, using Nelson's[8] colorimetric method to determine the reducing sugar produced by the action of the amylase upon the starch substrate.

For the determination of the serum lipase, a titrimetric method recently described by Alper[9] was used. This employs a tributyrin substrate and requires one hour's incubation. Units of lipase activity are expressed as milliliters of 0.1 N NaOH required to neutralize the fatty acids produced by the action of the lipase upon the tributyrin substrate.

RESULTS

The results of these studies have been analyzed according to postoperative enzyme changes in each of the first three groups, and also according to significant enzyme changes found in certain individual patients.

The mean preoperative serum amylase value for the first three groups of patients was 99.3 plus or minus a standard deviation of 37.7 units. Thus, the 95 per cent confidence limits for the preoperative group are 24 to 175 units. The random error of measurement computed from 81 duplicate determinations from individual patients was ±5.0 units[10] (Fig. 1).

Postoperative variations in serum amylase in the 20 operations which were remote from the pancreas, including abdominal and non-abdominal procedures, and which were classified in groups I and II, behaved in a similar fashion. These two groups, therefore, were combined and graphed in Figure 1*a*. The interrupted horizontal line indicates the preoperative mean of the group. Each bar represents the mean of the change in serum amylase obtained in the individual patients during the postoperative period, that is, on days 1, 2, 4, and 6. The vertical line through each mean value represents the standard error. The reference asterisk and triangle beside several of the bars indicates at what level the mean of the group is statistically different from zero according to the *t* test.[10] Postoperatively, a consistent moderate depression of serum amylase was noted, which was statistically significant on the second postoperative day

Figure 1*b* depicts changes in serum amylase following the 23 operations in close proximity to the pancreas, but involving no known direct pancreatic trauma, as classified in group III. The mean of the change in serum amylase was moderately, but significantly, elevated on the first postoperative day, followed by a depression on the second. However, the wide range of individual values, as depicted by a large standard error, indicates that the group was heterogeneous. In the majority of the 23 patients in this group, a slight

depression in amylase of a similar order of magnitude to that in groups I and II was found. However, in five of the 23 patients, significant elevations of serum amylase occurred, well beyond the 95 per cent confidence limits of normality for the preoperative group. These patients presented values above 200 units on the first postoperative day, as shown in Table 1. Two of these five showed values about 400 units. These patients were included in the analysis, however, since their operations and postoperative courses were no different from those of other patients in this group. They will be discussed individually later.

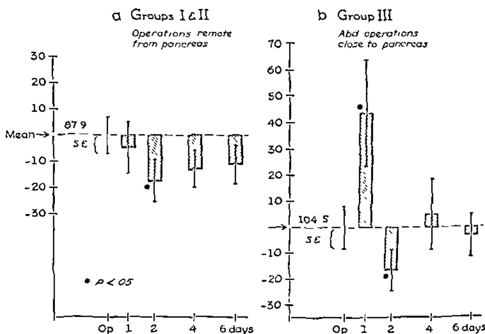


Fig 1.

The results of the serum lipase studies are graphed in Figure 2 in a fashion similar to the amylase results. Groups I and II are combined in one graph and group III is depicted in the other. The mean of the changes and their standard errors, as well as their statistical significance according to the t test, are presented. The mean preoperative serum lipase for the first three groups of patients, determined by Alper's method, was 1.15 plus or minus a standard deviation of 0 22 ml. 0.1 N NaOH. Thus, the 95 per cent confidence limits for the preoperative groups are 0.71 to 1.59 ml The random error of measurement computed from 28 duplicate determinations from individual patients was ±0.11 ml.[10]

In all
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cally sig—
and by the sixth postoperative day, the lipase was just beginning to return to normal.

In Table 1 are recorded serum amylase values for nine patients who presented one or more elevations of amylase postoperatively. There were no serum lipase elevations above the limits of normality in any patient, not even concomitantly with rises in amylase, with the method employed. None

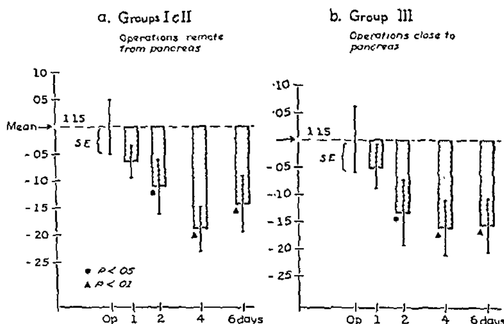


FIG. 2.

*Table 1. Serum Amylase Changes in Individual Patients**

NO	GROUP	SEX	OPERATION	PREOP.	DAYS POSTOP. 1	2	4	6
1	III	M	Cholecystectomy	75	111	103	*312*	181
2	III	M	Cholecystectomy	142	*253*	108	*216*	160
3	III	M	Subtotal gastric resection	113	*242*	100	193	104
4	III	M	Cholecystectomy and choledochostomy	165	*400*	167	*209*	192
5	III	F	A Exploratory laparotomy	68	65	39	39	56
			B Partial esophagectomy, partial gastrectomy	103	*480*	159	126	81
6	IV	F	Subtotal gastric resection	128	*322*	156	156	118
7	IV	M	Total gastrectomy, splenectomy, transverse colon resection, partial pancreatectomy	130	*425*	169	139	160
8	IV	M	Cholecystectomy	153	*815*	*337*	*249*	82
9	IV	M	Right thyroidectomy	156	*282*	154	98	94

* Serum amylase values above 200 units (which is above the 95 per cent limit of normality in this study) are italicized.

Although most authorities feel that the serum amylase must be above 300 to 500 units before the diagnosis of pancreatitis can be considered definite, cases of acute pancreatitis proved by autopsy or surgery have occurred with normal or only moderate elevations of amylase.[2,4] Therefore, all patients with amylase activity above 200 units, which is above the 95 per cent confidence limits of normality for the preoperative group, are recorded in this table.

The first five of these nine patients recorded were previously mentioned as being eligible for group III. Two patients (Nos. 1 and 2) had cholecystectomies during which the pancreas was not known to be manipulated or traumatized in any way. Patient No. 3, who underwent a subtotal gastric resection, and patient No. 4, who underwent cholecystectomy and choledochostomy, experienced no more manipulation of the pancreas than is usually necessary with these operations.

The last patient from group III who displayed an elevated serum amylase postoperatively is No. 5, who underwent a partial esophagectomy and partial gastrectomy for an esophageal carcinoma during which there was no known manipulation of the pancreas. Two weeks earlier, after an exploratory laparotomy, she displayed a moderate depression of serum amylase postoperatively. Amylase values for both operations are recorded.

The other four patients listed in Table 1 belong to group IV since, as mentioned earlier, they do not fit into any of the first three groups.

In two patients, the pancreas was directly traumatized. One (No. 6) underwent a subtotal gastrectomy and cholecystostomy for a gastric malignancy which had infiltrated into the head of the pancreas and had to be dissected away The other patient (No. 7) also had an infiltrating gastric malignancy, which required total gastrectomy, splenectomy, partial pancreatectomy, and resection of the transverse colon for its entire removal.

The highest amylase recorded was in patient No. 8, after a cholecystectomy. However, during the operation, the gallbladder was opened and stones and bile were spilled into the lesser peritoneal sac. Studies of Thistlethwaite and Hill[11] showed that whereas bile injected into the pancreatic ducts of dogs may cause an elevation of serum amylase, bile injected into the pancreatic parenchyma does not. This work would not substantiate the possibility that the patient's high amylase was caused by pancreatic irritation from bile. The patient's postoperative course was uncomplicated and free from any signs or symptoms of pancreatitis in spite of an amylase of 815 the first postoperative day and a gradual return to normal by the sixth day.

Another patient (No. 9) displayed a moderate elevation of amylase the first day after a right thyroidectomy for toxic goiter. This operation was felt to differ from the other non-abdominal procedures since thyroidectomy is the only operation outside of the abdomen after which fatal pancreatitis, actually several cases, has been reported.[12,13]

Maximal elevations of serum amylase in all patients except No. 1 occurred on the first postoperative day. The elevations in amylase observed after the two operations involving direct pancreatic trauma (Nos. 6 and 7) were no higher and were maintained over no longer a period than after three of the seven procedures involving no direct trauma (Nos. 4, 5, and 8). This points out the fact, mentioned by earlier writers, that trauma to the

pancreas cannot explain all of these postoperative elevations in serum amylase.

In this study, the enzyme changes could not be correlated with the anesthesia given or with the opiates used. It is impossible, however, to separate the effect of these agents from that of the operative procedure itself.

SUMMARY AND CONCLUSIONS

Forty-seven patients undergoing operations were studied with respect to alterations in serum amylase and serum lipase in the postoperative state.

In the majority of these patients, a moderate and consistent depression of serum amylase and lipase took place, but the values for the most part were within the 95 per cent confidence limits of normality.

Elevations of serum amylase above 200 units were shown to occur in an *occasional postoperative patient in whom no clinical evidence of pancreatitis* was found. This was especially true after operations in the vicinity of the pancreas even though no direct pancreatic trauma was known in most cases. The cause and significance of these elevations is not clear.

From our data, it would seem that the postoperative elevations in serum amylase are no more likely to occur after abdominal operations remote from the pancreas than following operations outside the abdomen.

There were no significant elevations in serum lipase concomitant with those in amylase when employing a method for the lipase determinations recently described by Alper.

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METABOLIC ALTERATIONS IN SURGICAL PATIENTS*

V. Cause and Management of Hyperchloremic Acidosis Following Ureterosigmoidostomy

LESTER PERSKY, HARVEY KRIEGER, STANLEY LEVEY, AND WILLIAM E. ABBOTT

Transplantation of the ureters into the large bowel has been the subject of much clinical and laboratory investigation.[1-6] The well documented electrolyte alterations which occur periodically in approximately 70 per cent[7] of the patients undergoing the procedure of ureterosigmoidostomy have been labeled hyperchloremic acidosis. Clinically, these patients show anorexia, nausea, vomiting, dizziness, dehydration and often fever. Electrolyte determinations reveal an elevated serum chloride, lowered carbon dioxide combining power and occasionally hypokalemia,[8] and decrease in serum calcium.[9] In an attempt to clarify the metabolic alterations which may occur following this surgical procedure, a study was undertaken utilizing metabolic balance techniques. To our knowledge, this investigational tool has not been applied previously to disorders of this type.

METHODS AND RESULTS

In the interest of brevity we shall describe the results obtained from two of the patients with this disorder studied on the Metabolic Division of the University Hospitals of Cleveland. The techniques for collection, measurement, and chemical analysis have been described, in detail, in a previous publication.[10] The patients were studied for periods of eleven and twenty days respectively. Since the urine was passed from the colon mixed with feces, the mixture was analyzed as one specimen except in case II where, during certain periods, the urine was diverted from the colon by means of nephrostomy tubes, permitting individual analysis of urine and feces.

Balance studies were carried out for water, chloride, sodium, potassium and nitrogen Only water, sodium, and chloride balances are presented in the charts since they demonstrate the most significant alterations The complete series will be reported in a subsequent publication [11] In the following charts the intake, output, and balance are plotted on the ordinate; the time is plotted on the abscissa. The intake is represented by the height of the column above the horizontal zero line, and the output is plotted downward from the top of the intake column Negative balance is indicated by the extension of the column below the zero or horizontal base line, while the white area above this line represents positive balance

Case I. M C. (Fig 1), a 42 year old woman, was studied approximately four months after a total cystectomy and ureterosigmoidostomy had been performed for squamous cell carcinoma of the bladder During the four month interval she had frequent episodes of dizziness, nausea, and vomiting. Physical examination was negative except for marked obesity. The initial plasma chloride, sodium, and potassium concentrations were 106, 150,

* From the Department of Surgery, Western Rese
and the University Hospitals of Cleveland, Cleveland
grants from the National Institutes of Health, U S
Elizabeth Severance Prentiss Foundation, and The Baxter Laboratories, Inc, Morton Grove, Illinois.

and 39 mEq. per liter respectively, CO_2 combining power was 49 volumes per cent (22.1 mEq. per liter) and blood urea nitrogen was 35 mg. per 100 ml. Preoperative [illegible] with evidence of chronic [illegible] without hydronephrosis.

During the first four study days the patient was given a constant daily diet having the following *electrolyte composition:* sodium 123 mEq., potassium 60 mEq., and chloride

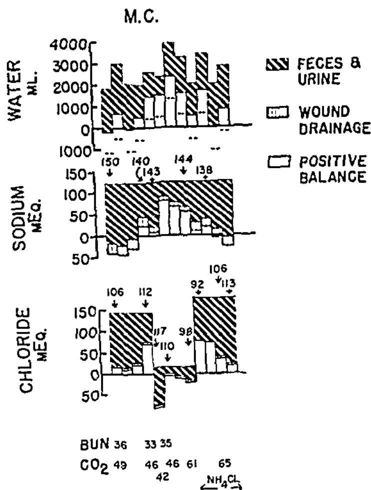


Fig. 1 Water, sodium and chloride balance studies in patient M C. The broken lines below the solid columns in the charting of the water balance represents the approximate amount of insensible fluid lost Plasma concentrations of sodium and chloride, in mEq. per liter, are shown above the respective balance charts for these ions Blood urea nitrogen (*mg per 100 ml*) *and carbon dioxide combining power (volumes per cent) are listed* at the bottom of the figure

145 mEq The diet contained 79 grams of protein, 54 grams of fat, and 178 grams of *carbohydrate yielding an intake of 1514 calories. During this period the fluid intake* was 2000 to 3000 ml. per day. The chloride balance was positive throughout this four day period, increasing from 15 mEq on the first day to 70 mEq. on the fourth day. The plasma chloride concentration increased from the admission value of 106 to 113 mEq. per liter, during which time a cumulative four day positive balance of 117 mEq. of *chloride* resulted A negative sodium balance of 25 to 50 mEq per day occurred, and the plasma sodium concentration fell from 150 to 140 mEq. per liter. There was a four day cumulative deficit of 96 5 mEq of sodium. An estimated cumulative fluid deficit of 2800 ml. occurred during this high chloride regimen.

During the next four days the intake of sodium, potassium, protein, fat and carbohydrate were kept at essentially the same levels while the chloride intake was decreased to one-tenth of its previous value The chloride balance became negative with this program and the plasma chloride concentration fell from 117 to 92 mEq per liter Sodium balance became positive (5 to 85 mEq. per day for a four day cumulative balance of 214 mEq.) while the plasma concentration increased slightly

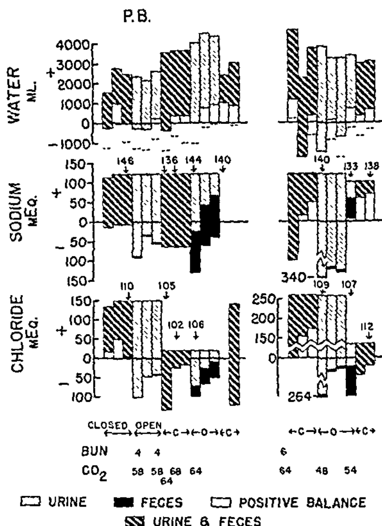


Fig 2 Water, sodium and chloride balance studies in patient P.B. The broken lines below the solid columns in the charting of the water balance represents the approximate amount of insensible fluid lost Plasma concentrations of sodium and chloride, in mEq. per liter, are shown above the respective balance charts for these ions Blood urea nitrogen (mg. per 100 ml) and carbon dioxide combining power (volumes per cent) are listed at the bottom of the figure The opening and closing of nephrostomy tubes *is shown* below the figure for chloride balance.

The patient was then given the previously described high chloride diet for the next

period ... sodium were excreted similar to that seen during the first four study days A cumulative positive chloride balance of 208 mEq occurred during this four day study period during which time the plasma chloride concentration increased from 92 to 113 mEq per liter.

Although the amount of ... there still was a cumulative four day positive sodium balance

... The acido- ... as given by

... would have occurred if the high chloride intake ...

Case II. P.B. (Fig. 2), a 60 year old woman, had a vulvectomy in 1951 for carcinoma of the vagina. In 1953, because of local recurrence, it was necessary to do a total vaginectomy and cystectomy *with implantation* of the ureters into the sigmoid colon. Four days ... because of temporary obstruction at the site of the

... limits prior to surgery and showed pelviectasis following surgery ... studied six weeks after operation. This study demonstrated the effects of having the nephrostomy tubes alternately closed and then opened during 3 day periods. The patient *had a daily chloride intake* of 150 mEq. during the first six days. When the tubes were closed and the urine drained into the sigmoid colon, permitting reabsorption of some *solutes, the chloride balance became positive and* the plasma chloride concentration ... 110 ... per liter. When the nephrostomy tubes were opened so that the ... chloride ... er. Upon ... moderately large chloride loss continued. In spite of the continued, although ... chloride, the plasma concentration increased (102 to 106 mEq. per liter), probably owing to an ... in a reduction of the extracellular fluid volume. When the

DISCUSSION

A greater rate of absorption of chloride than the sodium ion from the large intestine has been demonstrated in humans and in the experimental animal.[12] The data on P.B., the second patient, demonstrate the correction of acidosis by temporarily diverting the urine from the colon by means of nephrostomy tubes Other investigators have shown that the acidosis can be corrected by prolonged use of rectal tubes. In both situations tube drainage reduced the reabsorption of urinary solutes (especially chloride) to minimum.

In the present study, during acidosis, retention of chloride occurred, and the cumulative balance of this ion was positive. During this time large sodium losses developed, usually resulting in a negative sodium balance. *This negative sodium balance predisposes to the* development of dehydration, since the continuous loss of sodium is always accompanied by a loss of water. Case I, M.C., showed a negative water balance of approximately 850 ml. per day during *the periods of high chloride intake* if a daily insensible fluid loss of one liter is calculated in the balance. P.B., case II, showed a loss of about 900 ml. per day during a similar period. Dehydration results in a more concentrated urine being *presented to the absorbing* mucosa of the colon, and this, in turn, causes more chloride to be absorbed, both in the form of sodium chloride and ammonium chloride. Though formation of ammonia by the kidneys may be restricted *in the presence* of

During the next four days the intake of sodium, potassium, protein, fat and carbohydrate were kept at essentially the same levels while the chloride intake was decreased to one-tenth of its previous value. The chloride balance became negative with this program and the plasma chloride concentration fell from 117 to 92 mEq. per liter. Sodium balance became positive (5 to 85 mEq. per day for a four day cumulative balance of 214 mEq.) while the plasma concentration increased slightly.

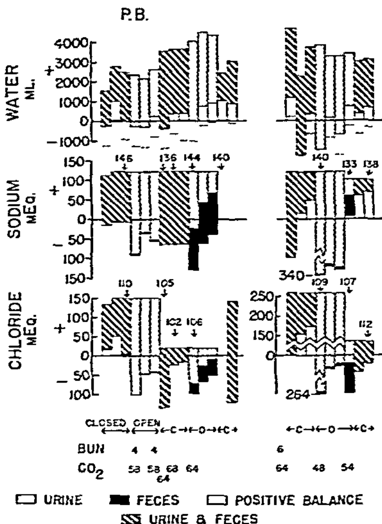


Fig. 2. Water, sodium and chloride balance studies in patient P.B. The broken lines below the solid columns in the charting of the water balance represents the approximate amount of insensible fluid lost. Plasma concentrations of sodium and chloride, in mEq. per liter, are shown above the respective balance charts for these ions. Blood urea nitrogen (mg. per 100 ml.) and carbon dioxide combining power (volumes per cent) are listed at the bottom of the figure. The opening and closing of nephrostomy tubes is shown below the figure for chloride balance.

The patient was then given the previously described high chloride diet for the next four days. In addition, she was given two grams of ammonium chloride per day. This increased the chloride intake by 57 mEq. per day over that given during the first four day period. A positive chloride balance again occurred, and progressively larger amounts of sodium were excreted similar to that seen during the first four study days. A cumulative positive chloride balance of 208 mEq. occurred during this four day study period during which time the plasma chloride concentration increased from 92 to 113 mEq. per liter.

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positive sodium b
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the condition of the patient prior to each of these periods. The patient
towards hyperchloremic acidosis when the first high chloride diet was started. The acidosis had been corrected prior to the second time the high chloride regimen was given by an intervening period of low chloride intake. It is apparent from the trend of the sodium balance that sodium and water losses similar to those seen in the first four day period would have occurred if the high chloride intake had been continued.

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DISCUSSION

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renal disease, the absorption of ammonium chloride is equivalent to presenting the body with free chloride ions, since the ammonium ion is converted to urea. Thus, in such patients the normal physiologic compensatory mechanisms, i.e, the existence of a concentrated urine in the presence of dehydration, formation of ammonium ion, and detoxification of ammonium, all combine to intensify the hyperchloremic acidosis

D'Agostino, Leadbetter and Schwartz[13] have shown, in isolated loops of bowel, that there is an exchange of bicarbonate for chloride. Their work suggests a mechanism for increasing the chloride ion in the body of these patients. This exchange should lead to a retention of chloride and a loss of both sodium and bicarbonate Parsons,[12] in a similar preparation, has demonstrated that there is a more rapid absorption of chloride than sodium. The loss of sodium is in all probability due to the excretion of this ion with chloride since many of these patients show a reduction or inability of the kidneys to form ammonia. Such patients thus cannot conserve fixed base by substituting the ammonium ion for sodium (or potassium, calcium and magnesium).

However, it is possible to hypothesize that even in the presence of normal kidneys the loss of sodium and water, as well as a high chloride intake and the reabsorption of this ion from the bowel, could lead to a hyperchloremic state. Hyperchloremic acidosis has also been easily induced in dogs with vesicosigmoidostomies without kidney disease by similar alterations in the electrolyte intake and following dehydration.[14] The fact that hyperchloremic acidosis could be induced by giving a larger intake of chloride than fixed base (as would usually occur in the ordinary diet), or be relieved by increasing the intake of sodium and potassium associated with anions (bicarbonate, lactate or citrate) that can be metabolized to water and carbon dioxide, demonstrates that the role of the kidneys is of secondary impor- [illegible]

be corrected, in most instances, by the proper dietary and fluid regimen. In the two patients discussed here there was, however, some degree of antecedent renal damage. It is apparent that kidney disease facilitates the development of hyperchloremia Thus, while kidney damage might not be essential for the development of this syndrome, its presence enhances the likelihood of acidosis and makes mandatory proper care and management.

Our studies showing retention of chloride and loss of sodium and water have led to the adoption of a regimen of adequate hydration, limitation of the chloride intake to approximately 30 to 70 mEq. daily, and the administration of supplementary base in the form of sodium bicarbonate and potassium citrate Ambulatory management under these conditions is simple, and has been adequate, in most instances, to avoid periodic episodes of acidosis.

SUMMARY

The results of the metabolic balance studies on two patients with ureterosigmoidostomy are presented during periods in which hyperchloremic acidosis was induced and relieved by altering the electrolyte intake. It was found that during acidosis these patients had a positive chloride balance and a negative sodium and water balance. The mechanisms leading to the devel-

opment of hyperchloremic acidosis, along with appropriate therapy to combat it, have been discussed.

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METABOLIC ALTERATIONS IN SURGICAL PATIENTS*

VI. The Effect on Weight and Nitrogen Balance of Providing Varying Caloric Intakes by Intravenous Carbohydrate, Fat and Amino Acids in Gastrectomized Patients

WILLIAM E. ABBOTT, JOHN H. DAVIS, JERREL W. BENSON, HARVEY KRIEGER, AND STANLEY LEVEY

There seems to be no great unanimity of opinion concerning the feasibility or advisability of attempting to prevent or minimize the nitrogen wasting which occurs early after surgical trauma. Although some workers[1, 2] have expressed the belief that little can be accomplished in the immediate postoperative period to minimize the weight and nitrogen losses, others[3-7] have

* From the Department of Surgery, Western Reserve University School of Medicine, and University Hospitals of Cleveland, Cleveland, Ohio. This study was supported by grants from the National Institutes of Health, U. S Public Health Service (G3408), the Elizabeth Severance Prentiss Foundation, and The Baxter Laboratories, Inc., Morton Grove, Illinois.

reported that by providing an adequate nutritional intake these deficits can be substantially reduced. During the convalescence following infection, injury or a surgical procedure, repair and restoration are accomplished by the formation of new tissue, which depends largely upon providing sufficient amounts of utilizable nitrogen and calories. Although the energy requirements of the post-traumatized individual are increased,[8, 9] the specific dietary needs of each patient can only be roughly estimated. Few patients will take a nutritionally adequate diet in the first four to seven days following major surgery. If force-fed or given nutrients by tube, vomiting, intestinal distention or diarrhea often occur. In the past when intravenous alimentation was employed, the patient usually received 400 to 800 calories per day and thus rarely had the benefit of an adequate intake of nutrients. With the improvement of protein hydrolysates and addition of concentrated carbohydrate solutions, and intravenous fat emulsions, it is now possible to provide a parenteral regimen adequate in both calories and utilizable nitrogen The purpose of this study was to determine the effect of various caloric and nitrogen intakes on the magnitude of the nitrogen deficits which occur in patients following operative trauma.

METHODS

Metabolic studies were performed on 28 patients undergoing gastric operations. Twenty-three of these patients had peptic ulcer and five had malignant lesions of the stomach These patients were maintained on the Surgical Metabolic Division of the University Hospitals of Cleveland, where postoperative studies were carried out for 10 to 15 days and, in most instances, preoperative studies were also undertaken for two to ten days. All of these patients had a comparable degree of operative trauma and were maintained exclusively by intravenous alimentation during the first five days of the study (day of operation and the first four postoperative days). It was decided to evaluate the results of this period since the parenteral regimen employed in each patient was kept as constant as possible during these five days. While the regimen did not vary greatly in each patient it was purposely altered from one patient to another so that we might determine the effects of giving various caloric and nitrogen intakes on the nitrogen balance during the immediate postoperative period. Aliquots of all parenteral and oral foods and all excreta (urine, feces, gastro-intestinal and wound drainage, emesis and sputum) were analyzed, in duplicate, for sodium, potassium, chloride and nitrogen Although the results of the balance data of sodium, potassium and chloride are not included in this report they were considered in interpreting the alterations in nitrogen balance. Since blood was given to these patients only on the day of operation in amounts calculated to replace that lost, it was not considered in the metabolic balance calculations.

The information obtained from the preoperative study and additional studies which were conducted after the reported five day period were also used in interpreting the metabolic changes and classifying the patients into the various groups discussed. The complete data will be presented elsewhere.[10]

In order to obviate any effect that might occur from an inadequate vitamin intake these patients were all given at least five times their normal vitamin requirements daily. The parenteral nutrients employed were 5 per cent protein hydrolysates, 5, 10 or 20 per cent dextrose, 10 per cent fructose or

invert sugar solutions and a 15 per cent fat emulsion containing 4 per cent dextrose.* The methods of carrying out the balance study and performing the chemical analyses have been previously described.[11]

RESULTS

In Tables 1, 2, 3, 4 and 5 are given the sex, age, weight and the caloric and nitrogen intake per kilogram per day of each of the subjects for the day of operation and the first four postoperative days. The total cumulative five day nitrogen deficit or gain is shown in the last column. The patients included in Tables 1, 2 and 3 were all men, in Table 4 all women, and in Table 5, two men and three women. The patients listed in the first four tables were operated on for peptic ulceration, while those in Table 5 had comparable operations although slightly more extensive for malignant tumors of the stomach.

Table 1. Nitrogen Balance Studies in Five Patients Who Had Moderate Caloric and Nitrogen Intakes

PATIENT	SEX	AGE	WT. KG.	NUTRITIONAL INTAKE PER KG. OF BODY WT. PER DAY		FIVE DAY CUMULATIVE NITROGEN BALANCE
				CALORIES	NITROGEN	
J.C.	M	50	52	65	0 32	−13 5
R.S.	M	43	45	45	.44	−29.0
P.W.	M	60	60	40	23	−25.9
M.U.	M	55	60	30-40	.21	−33 9
C.S.	M	62	51	20-22	.22	−9.2

In Table 1 the results are shown that were obtained from the study of five men who were given minimal to what was thought to be adequate caloric and nitrogen intakes. These patie[illegible]
nitrogen deficit of 21.9 gm. or an average [illegible]
In this group, two of the men (R.S. and M.[illegible]
rior gastric ulcers. In both instances the ulcer had perforated through the superior-posterior gastric wall and penetrated into the pancreas and liver, causing a marked inflammatory reaction. Postoperatively, their temperatures fluctuated between 38° and 38.7°C., subsiding to normal near the end of the fifth day. In these two patients who had a fairly persistently elevated body temperature during the first four postoperative days the average cumulative nitrogen deficit was 31.5 gm. as compared to 16.2 gm. for the three afebrile patients in this group.

In Table 2 the results obtained from a study of seven men undergoing a

* We are indebted to [illegible] ins (Soluzyme with ascorbic acid) and [illegible] Baxter Laboratories, Inc, Morton Grov[illegible] intravenous solutions. 5 and 10 per cent dextrose, 10 per cent fructose and 10 per cent invert sugar (Travert), as well as a 5 per cent plasma hydrolysate (Travamin) in 10 per cent invert sugar (Travert), Mead Johnson and Co, Evansville, Indiana, for 10 per cent fructose (Levugen) and a 5 per cent casein hydrolysate (Amigen) in a 10 per cent fructose (Levugen) solution

comparable operative procedure are shown. These patients were given a slightly lower caloric and a minimal nitrogen intake. The five day cumulative nitrogen deficits ranged from 18 2 to 44.6 gm. with an average cumulative nitrogen deficit of 35.1 gm. The greatest loss (44.6 gm.) was noted in T.M., a healthy, vigorous, 42 year old man, weighing 79 kilograms, who received the smallest caloric and an inadequate nitrogen intake. The smallest loss (18 2 gm.) was seen in W.Mc. who, although not malnourished, had lost some weight preoperatively and thus would not be expected to show a

Table 2. Nitrogen Balance Studies in Seven Patients Who Had a Suboptimal Caloric and Nitrogen Intake

PATIENT	SEX	AGE	WT. KG.	NUTRITIONAL INTAKE PER KG. OF BODY WT PER DAY		FIVE DAY CUMULATIVE NITROGEN BALANCE
				CALORIES	NITROGEN	
H.C.	M	24	51	25	0 183	−34.5
C A	M	61	72	19-24	19	−33 7
R H.	M	43	53	27	174	−39 1
A.L.	M	38	72	20-24	17	−36 8
P.W	M	44	61	19-24	10	−39 1
W.Mc	M	49	61	20	13- 17	−18.2
T M	M	42	80	12	0- 15	−44 6

metabolic response as vigorous as the others in the group. It will be noted (Table 3) that the five men given inadequate caloric and zero nitrogen intakes showed cumulative five day nitrogen deficits of from 49.5 to 69.7 gm. or an average deficit of 60.1 gm. (equivalent to a loss of 12 gm. of nitrogen per day for the first five days).

In Table 4, the results obtained from five women who underwent a subtotal gastrectomy for peptic ulceration are shown. The first three patients were malnourished and although their caloric and nitrogen intakes were suboptimal they exhibited an average five day cumulative nitrogen deficit of only 4.74 gm or an average daily negative nitrogen balance of 0 95 gm.

Table 3 Nitrogen Balance Studies in Five Patients Who Had an Inadequate Caloric and Zero Nitrogen Intake

PATIENT	SEX	AGE	WT KG	NUTRITIONAL INTAKE PER KG OF BODY WT PER DAY		FIVE DAY CUMULATIVE NITROGEN BALANCE
				CALORIES	NITROGEN	
F.U.	M	19	42	29	0	−50 9
D B	M	42	58	20	0	−69 7
T B.	M	37	77	10	0	−49 5
M.T.	M	62	69	10	0	−63 9
E.W.	M	61	65	10	0	−66.7

The remaining two women in this group were moderately well nourished and showed only modest nitrogen losses (an average five day cumulative nitrogen deficit of 12.8 gm.).

The results obtained in the study of five patients undergoing gastrectomy for malignant tumor of the stomach are shown in Table 5. M.B. received no nitrogen but was given 16 calories per kilogram per day. She had a cumulative five day nitrogen deficit of 28.0 gm. or an average loss of 5.6 gm. of nitrogen per day. While this loss is larger than that of any other patient in

Table 4. Nitrogen Balance Studies in Five Malnourished Women on a Moderate Caloric and Nitrogen Intake

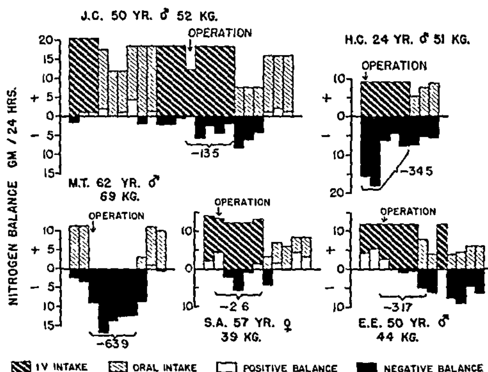
PATIENT	SEX	AGE	WT. KG.	NUTRITIONAL INTAKE PER KG. OF BODY WT. PER DAY		FIVE DAY CUMULATIVE NITROGEN BALANCE
				CALORIES	NITROGEN	
S.A.	F	57	39	20	0.32	−2.63
A K.	F	78	50	25	24	−0.10
M G.	F	78	43	14-28	.28	−11 2
S W.	F	42	47	30	128	−16 6
M C.	F	29	58	20	.21	−8 9

this group it is considerably less than that seen in the well nourished men (Table 3) who had a similar intake, undergoing a comparable operation. This difference in the metabolic response to a comparable trauma occurred not only because this patient (M.B.) was malnourished but also because women respond less vigorously to surgical operations than men. The second and third patients (Table 5) were malnourished women with adenocarcinoma and sarcoma of the stomach, respectively. They received fairly adequate caloric (31 to 46 cal. per kilogram) and nitrogen (0 3 to 0.45 gm. per kilogram) intakes and had an average five day cumulative nitrogen deficit of 4.27 gm. or an average loss of 0.85 gm. per day. The fourth and fifth cases, who were undernourished men with adenocarcinoma of the stomach, were maintained on a higher caloric and minimal nitrogen intake, and showed an average five day cumulative nitrogen deficit of 8.94 gm.

Table 5. Nitrogen Balance Studies in Five Malnourished Patients with Malignant Tumors of the Stomach

PATIENT	SEX	AGE	WT. KG.	NUTRITIONAL INTAKE PER KG OF BODY WT. PER DAY		FIVE DAY CUMULATIVE NITROGEN BALANCE
				CALORIES	NITROGEN	
M.B.	F	63	36	16	0	−28.0
J.K.	F	65	36	46	0 45	+3 36
O.R.	F	52	50	31	30	−11 9
N.F.	M	60	47	55	.31	−14 7
E E.	M	60	45	54	.27	−3.17

The results of the nitrogen balance studies for five representative patients are shown in Figure 1. One patient was selected from each of the aforementioned groups (Tables 1-5). These balance studies are plotted by showing the scale for intake and the balance per 24 hours on the ordinate and the scale for time on the abscissa. The entire intake is plotted from the horizontal zero line upward and the total output plotted downward from the top of the intake column. Positive balance is shown as the white area above the



days are shown below each of the balance charts.

zero line and the degree of negative balance is represented by the extent the black columns project below this line. The periods of oral intake are designated by black diagonal lines on white and the periods of total intravenous feeding are indicated by white diagonal lines on black. The charts illustrate that during the immediate postoperative period (5 days) the deficits of nitrogen are minimized when larger intakes of nutrients are provided. After the day of operation and the first four postoperative days it should be noted that the nitrogen deficit increased when the nitrogen (and caloric) intake was reduced This is apparent in the graphs of patients J.C., S.A. and E.E. In contrast, M T showed a change from a marked nitrogen deficit to equilibrium when adequate calories and nitrogen were provided.

DISCUSSION

The results of this study indicate that although a negative nitrogen balance was not prevented postoperatively in the previously healthy patient,

the deficits could be minimized by providing a fairly adequate caloric and protein intake. In malnourished patients, a significantly positive nitrogen balance was often obtained preoperatively by giving adequate feedings. During this period a comparable effect on the nitrogen balance was attained whether calories and nitrogen were administered orally or intravenously. It is apparent from these studies that the weight loss and nitrogen deficit could be substantially reduced by the administration of adequate calories and nitrogen postoperatively and this was especially evident in the undernourished patient. Since the nutritional intakes given to the patients in this study were often suboptimal it should also be remembered that the weight and nitrogen losses might well have been further reduced if the caloric and nitrogen intakes had been comparable to those recommended by Rhoads[12] (40 calories per kilogram per day and 0.40 gm. of nitrogen per kilogram per day).

In other studies[13] patients who received forced oral feedings of a very high caloric and nitrogen diet for the first few days after a severe injury had so much distress and interference with recovery that such a regimen does not seem warranted. Conversely, several days or more of a deficient intake should be avoided. It has been emphasized[14] that when the caloric intake falls below the daily energy requirements extra protein is expended; thus, it seems apparent that larger amounts of nutrients should be supplied than have been generally employed in the immediate postoperative period.

Four patients, J.C., P.W. (Table 1), N.F., and E.E. (Table 5), received in addition to the carbohydrate and amino acid solutions 600 ml. of a fat emulsion intravenously (equivalent to 900 calories) for 6 to 16 days. While these four patients had no evident reactions to the administration of an intravenous fat emulsion the incidence of reactions in our series has been approximately 15 per cent. Since it has been shown[15] that an experimentally induced fever causes an increased excretion of nitrogen, thermogenic reactions associated with the administration of any parenteral solution will often negate the giving of added nutrients. Such an impression seems to be borne out by our studies.[10] No statement is justified on the basis of this limited study concerning the relative merits of fat as a nitrogen sparing agent. However, the caloric potential of fat emulsions is significantly greater (2 to 7 times per milliliter) than other parenteral fluids so that at present, when given in conjunction with protein hydrolysates and carbohydrates, such a regimen offers the best hope of meeting the nutritional demands of the average patient who is unable to take oral feedings.

While some controversy exists concerning the ability of the previously healthy individual to utilize protein immediately following an injury the evidence at present seems to favor the view that the administered nitrogen is utilized. The existence of a negative nitrogen balance during periods of protein alimentation does not indicate that protein is not being utilized. Some of the confusion exists due to the varied interpretations of the word "utilize." Some have intimated that the body does not utilize protein unless it is converted into hemoglobin, plasma or tissue proteins. Following severe injury the fact that not more than a few per cent of the total urinary nitrogen is due to the presence of amino acid nitrogen substantiates the view that administered protein or protein hydrolysates are utilized.[16] It is therefore apparent that they might be utilized to meet the energy requirements as well as for the formation of new tissue. In order that the latter be accom-

plished it is necessary to provide sufficient nutrients to supply the energy needs, as well as to give an adequate amount of protein for tissue synthesis. The work of Shaffer and Coleman,[17] and later Werner and his associates,[18] indicates that the diversion of administered protein into anabolic processes may be largely accomplished after the patients' energy requirements have been met. By the use of radioactive tracer techniques, Madden[19] has shown that following an acute injury (turpentine abscesses) dietary methionine-sulfur is incorporated into both tissue and plasma proteins at a normal or accelerated rate He further points out that the negative nitrogen balance exhibited following such an injury appears to be caused by increased rates of catabolism rather than an interference with anabolism. Thus, it appears that most of the ingested protein passes through the usual metabolic pool and that after injury the nitrogen consumed is not immediately spilled in *the urine after being burned for energy. Rather, it is incorporated into newly* formed tissue and plasma proteins which, in turn, are subsequently degraded and the metabolic end products are then excreted in the urine. The experimental studies of Madden[19] and of Madden and Clay,[20] as well as the clinical metabolic studies herein reported and those of other workers[3,7] dealing specifically with patients undergoing a subtotal gastrectomy, support the thesis that the maintenance of an adequate caloric and protein intake is preferable to the deficient regimens that are commonly employed today

CONCLUSIONS

These studies emphasize the fact that in the first six to eight postoperative days the usual dietary regimens employed are markedly insufficient in both calories and nitrogen and therefore lead to the unnecessary loss of nitrogen and body weight. Those patients who were given a deficient intake of nutrients during this period showed large weight and nitrogen losses, while those who received a more nearly optimal intake had minimal deficits. The malnourished patients derived the most benefit from adequate oral and *parenteral feedings in that they showed a maintenance or gain of body* weight and slight deviation from nitrogen equilibrium.

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THE EFFECT OF PARATHYROID HORMONE UPON SERUM LEVELS AND URINARY EXCRETION OF MAGNESIUM*

BROOKE ROBERTS, JOHN J. MURPHY, LEONARD MILLER, AND OTTO ROSENTHAL**

It is known that abnormally low levels of magnesium in the body may result in tetany.[1] It is also known that significant amounts of magnesium are found in bone and that parathyroid hormone has profound effects on bone metabolism. Calcium and magnesium are both divalent ions and have certain chemical similarities. Since it seemed possible that calcium and magnesium might be effected similarly by parathormone, the effect of the hormone on serum levels and urinary excretion of magnesium was studied in the dog.

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** We wish to express our thanks to the Eli Lilly Company for furnishing the Solution Parathyroid Extract used in these experiments We also wish to thank Dr. John Reinhold and Miss Nancy Newlin for their great help in the chemical determinations.

METHODS

Adult female mongrel dogs were used as the experimental animal. One group of dogs was subjected to total parathyroidectomy (with thyroidectomy) under pentobarbital anesthesia after base line studies had been made on the serum levels of magnesium, calcium, phosphorus, and on the urinary excretion of magnesium. Dogs in which the parathyroids had not been completely removed, as judged by the absence of the calcium response, were discarded. Urine was collected by means of an indwelling catheter leading to a rubber urinal bag which was strapped to the animal's abdomen. Twenty-four hour specimens were thus collected and determinations of serum magnesium, calcium and phosphorus were made at twenty-four or forty-eight hour intervals. The dogs were followed postoperatively with chemical studies until death, which usually occurred about the fifth day. During this time, the animals were offered the standard kennel diet and as much water as desired. In six dogs, the serum levels of the three ions and urinary magnesium excretion were studied. In an additional two animals, only the serum levels were followed.

In the two dogs used as controls, the thyroid glands were exposed but not removed.

Another group of dogs, after a similar period of base line studies of urine and serum, received daily injections of Solution Parathyroid Extract (Lilly) for five days Only two of six dogs in this group survived this treatment. Two dogs received 400 U S.P units* of parathormone daily while the other four received 200 units daily The two dogs that survived were allowed to recuperate for three days and then subjected to parathyroidectomy and are included in the former group

The calcium determinations were made by the method of Clark and Collip,[2] the phosphorus determinations by the method of Fiske and Subba-Row,[3] and magnesium was determined colorimetrically by means of a slight modification of the titan yellow method of Heagy.[4]

RESULTS

The eight dogs subjected to parathyroidectomy showed a progressive fall in serum calcium levels, as anticipated, and all showed at least temporary rises in serum phosphorus. Three dogs, however, showed a drop in their serum phosphorus after a temporary rise in spite of the continued fall of the calcium levels. In general there was a trend for the serum magnesium to drop (Fig. 1). This trend, however, was not wholly consistent or of great magnitude In the two dogs that had previously survived a course of parathormone and were then subjected to parathyroidectomy, major alterations in serum magnesium were seen (Fig 2). One of these developed a high serum magnesium prior to death, as did the dogs dying promptly after parathormone administration The other had an abnormally high serum level at the time of operation but this gradually dropped toward normal postoperatively. The serum magnesium of the other six dogs subjected to operation remained normal or slightly subnormal until death. The change in urinary

* One U S P. parathyroid unit is defined as one one-hundredth of the amount required to raise the calcium content of 100 cc of the blood serum of normal dogs 1 mg. within sixteen to eighteen hours after administration.

magnesium excretion following parathyroidectomy did not appear consistent or significant (Fig. 3).

Of the six dogs given parathormone daily for five days, four died within forty-eight hours of cessation of its administration. Death was apparently caused by the drug. The expected rise in serum calcium was seen in every case and was usually progressive. There was also a uniform rise in serum phosphorus levels up to a maximum of 9 mg. per cent in one dog. There was no significant change in the serum magnesium of any dog for the first three days of parathormone administration. Thereafter, all dogs showed a

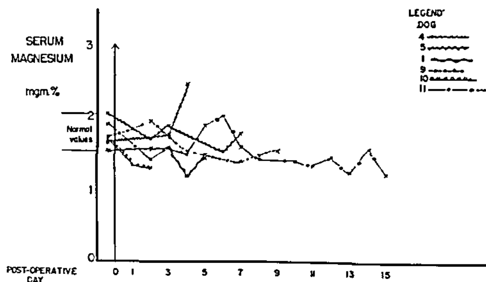


Fig. 1. Serum magnesium levels in dogs after parathyroidectomy.

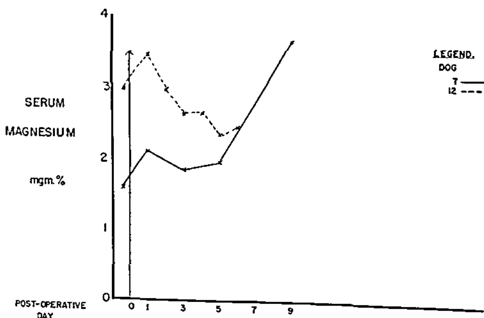


Fig. 2. Serum magnesium levels after parathyroidectomy in dogs having previously received parathyroid extract.

rise to abnormal levels usually on the fourth to sixth day (Fig. 4). Elevated blood urea nitrogen levels were found in the two animals in which this study was made at the time. A consistent finding in these six dogs was an increase in the urinary excretion of magnesium following injection of parathormone (Fig. 5). This effect was maximal by the third day and in some animals amounted to an increase of over 500 per cent. This increase was primarily due to an increased concentration of the ion in the urine rather than a diuresis After the third day, the excretion constantly decreased in spite of the continued use of the hormone. As the urinary excretion of magnesium began to fall, there usually was a corresponding increase in the serum level of this ion.

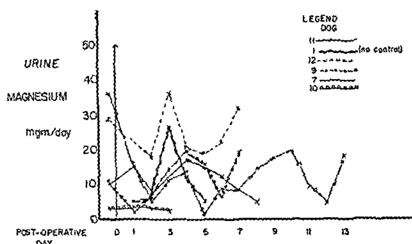


Fig 3 Urinary magnesium excretion in dogs after parathyroidectomy

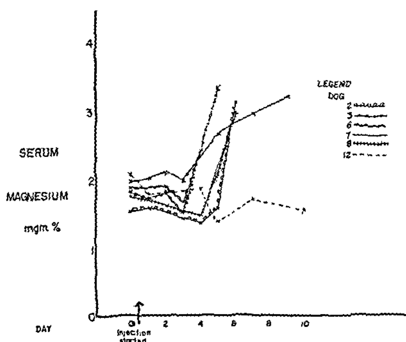


Fig. 4. Serum magnesium levels in dogs receiving parathyroid extract.

No significant changes were found in the blood or urine levels of these electrolytes in the control animals.

DISCUSSION

The administration of this preparation of parathormone appears to have a definite effect on magnesium metabolism. It causes a prompt and marked increase in urinary excretion of this ion. The subsequent fall in excretion may well be due to renal damage following use of this preparation.[5] The delayed rise in serum levels of magnesium may also be explained on this basis. Although we confirmed the finding of a diuretic effect of this preparation,[6] the diuresis played a minor part in the increase in excretion of magnesium and the increased concentration accounted for a greater part of the rise. Since the serum levels are not significantly altered during this period

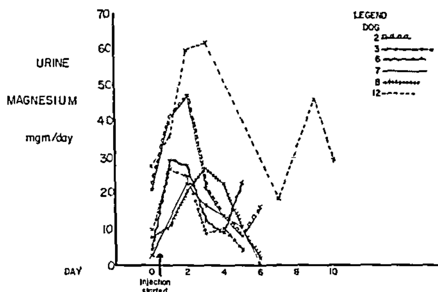


Fig 5. Urinary magnesium excretion in dogs receiving parathyroid extract.

of urinary magnesium loss, it would appear that this ion is mobilized from reservoirs within the body, possibly bone or muscle. It seems unlikely that the late rise in serum magnesium after use of parathormone is due to a non-specific antemortem change in cell membrane permeability with magnesium leaking from the cells. Such a rise is not found in the animals dying after parathyroidectomy nor is it ordinarily seen in humans prior to death.[7]

The increase in serum phosphorus noted after use of parathyroid extract has been shown to be due at least in part to a non-specific effect of the tissue extract[8] and may also be related to renal damage.[5]

If the effects on magnesium are direct effects of the hormone, it is surprising that we do not see greater converse effects following the removal of the parathyroid glands. It is possible, however, that if these dogs had had demineralization or cystic changes in their bones prior to removal of their parathyroids, more definite changes in the magnesium levels might have been seen.

The possible complicating effect of removing the thyroid with the para-

thyroid glands is not believed to be of consequence because of the short duration of the postoperative period

SUMMARY

1. The administration of Solution Parathyroid Extract to normal dogs results in increased urinary excretion of magnesium.
2. A delayed fall in urinary magnesium excretion followed by a rise in serum magnesium is then seen. These changes are probably due to renal damage by the preparation.
3. Parathyroidectomy in normal dogs has little effect on magnesium balance except for minor decreases in serum levels of this ion.

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THE INFLUENCE OF PROTEIN LEVEL ON THE VOLUME EXPANSION AND MAINTENANCE GIVEN BY DEXTRAN*

W. METCALF AND L. M. ROUSSELOT**

A number of macromolecular colloids, dextran, polyvinylpyrrolidone, gelatin, oxypolygelatin, and globin have been intensively investigated recently regarding their actual or potential usefulness as plasma expanders.[1,2] The comparative efficacy of these substances in relation to each other, and to standard solutions such as saline, glucose, plasma, and blood, have been determined in human subjects and experimental animals.[3-8] In general, it has been found that the volume expanding properties of a particular expander are determined by its average molecular weight and molecular weight spectrum.[9,10] However, the factors governing the magnitude of the

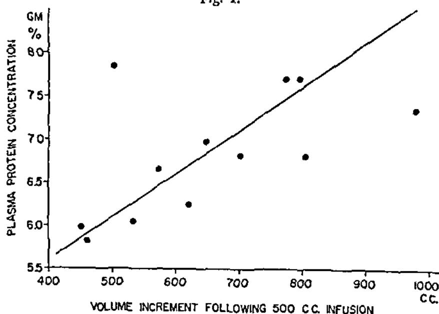
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** The authors gratefully acknowledge the technical assistance of Miss Sally Davis.

immediate plasma expansion and the expansion maintenance in any individual subject have not been particularly studied or emphasized.

In the original experiments in this laboratory the expansion obtained with a 500 cc. dextran infusion varied from 400 to 900 cc. The individual values correlated directly with the original protein concentrations. In a subsequent study with 1000 cc. infusions the same correlation was found to hold. In addition, the expansion maintenance over 24 hours in each individual, in both the 500 and 1000 cc. infusion series, was found to correlate directly with the original plasma protein concentration.

Fig. 1.



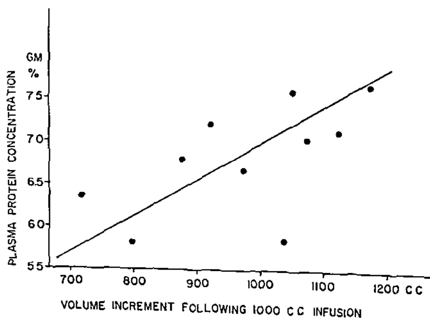
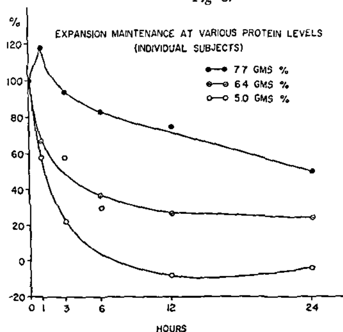


Fig. 2.

METHODS

Essentially normal surgical patients served as the test subjects. The received either 500 or 1000 cc. of 6 per cent dextran in normal saline* in 2

Fig 3.



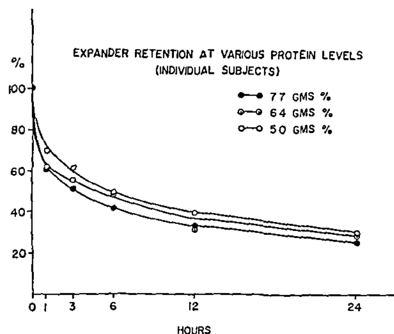


Fig. 4

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or 40 minutes. The original plasma volume was determined from a 10 minute specimen following injection of T-1824. The plasma volumes 10 minutes and 1, 3, 6, 12, and 24 ... were calculated from the changes i... nd in protein concentration. The ser... ...nined by a turbidimetric procedure[11] and the proteins, hemoglobins, and hematocrits by standard methods.

RESULTS

The plasma protein concentrations ranged between 5.0 and 7.8 grams per cent. The expansions obtained with the 500 cc. infusions varied from 400 to 900 cc., and with the 1000 cc. infusions from 700 to 1100 cc.

The correlation between the immediate postinfusion expansions and the original protein concentrations are shown in Figures 1 and 2. It may be noted that when the protein concentration is greater than 6.2 grams per cent in the 500 cc. series and 7.0 grams per cent in the 1000 cc. series, the expansion volume is greater than the volume infused; with proteins below these levels the expansions were less than the volumes infused.

The correlation of volume expansion maintenance over 24 hours with

Table 1.

NO. OF SUBJECTS	PROTEIN RANGE GM %	AVERAGE EXPANSION % OF VOLUME INFUSED						
		HOURS						24 HOUR
		0*	1	3	6	12	24	AVERAGE
3	5 00–5.99	93.1	61.5	45 0	26 8	11.5	3.0	20.0
9	6.00–6 99	97.8	71.2	56 3	41.3	39 1	18.1	37.0
8	7.00–7.99	111.3	83.6	64.4	54 9	60.1	32 2	55.0

* Actually 10 minutes after the infusion.

protein level in the individual subjects is very striking. Figure 3 shows the 24 hour expansion maintenance in subjects with original protein concentrations of 7.7, 6.4, and 5 0 grams per cent.

It is seen that the best maintenance is in the patient with the highest protein concentration. It may be noted, too, that in the patient with a protein level of 5.0 grams per cent, practically none of the 1000 cc. infused remained in the circulation after 6 hours. On the other hand the patient with the high protein still retained 800 cc. at 6 hours and 500 cc. at 24 hours.

The poor volume maintenance in the low protein subjects does not appear to be due to greater expander loss from the circulation. In Figure 4 the expander retention (amount of expander substance remaining in circulation) is shown for the same three experiments. The spread in expander retention is never more than 10 per cent. The spread in volume maintenance, however, between the low and high protein subjects is 60 to 70 per cent.

The relation between protein level and expansion maintenance noted for these three individuals also holds for the whole series. Table 1 shows the volume maintenance data grouped according to protein levels and averaged at the various intervals after infusion.

The spread of the average volume maintenance between the low and

high protein groups, although not as great as with the individuals at either extreme, is still 20 to 50 per cent. On the other hand, the spread of the average expander retention, as indicated in Table 2, is only from 4 to 8 per cent.

DISCUSSION

The only criterion for the efficacy of an expander is the volume expansion and maintenance obtained in relation to the volume infused. These experiments indicate that even in a group of "normal" subjects the response to a *given volume of expander can vary widely in both expansion and maintenance.*

From a theoretical point of view, the positive correlation of expansion and maintenance to original protein level noted here is paradoxical. Usually, when a hyperoncotic solution is introduced into the circulation the plasma volume is increased by the influx of water from the extravascular space until the concentration of the new substance becomes iso-osmotic with the plasma. The 6 per cent dextran solutions used were no doubt hypertonic to the plasmas in the experimental subjects with protein concentrations between 5.0 and 6.0 grams per cent. Yet, in these, fluid was not added to the circulation over and above the amount infused, rather, it was lost more rapidly and in greater amounts.

Table 2.

NO OF SUBJECTS	PROTEIN RANGE GM %	AVERAGE RETENTION % OF AMOUNT GIVEN						
		HOURS						24 HOUR
		0*	1	3	6	12	24	AVERAGE
3	5.00–5.99	89.9	74.1	61.9	51.8	40.9	28.8	41.1
9	6.00–6.99	83.0	70.3	56.7	48.4	38.5	27.6	40.2
8	7.00–7.99	88.9	73.1	63.8	54.9	44.6	35.5	49.0

* Actually 10 minutes after the infusion

It is interesting that the retention of the expander substance was almost identical in the three protein groups. In spite of wide variations in the fluid movement into or out of the circulation the curves of expander disappearance were parallel to each other with an over-all spread of only 10 per cent or less. The movement of the expander out of the circulation through the glomerular and body capillaries thus appears to be governed by the size and shape of its molecules and the physical properties of the pores in the capillary bed.

From a practical standpoint the findings in this group of experiments indicate that it would be difficult, if not impossible, to predict with any accuracy the volume expansion obtainable with these solutions in any given individual without prior knowledge of his protein concentration and plasma volume.

These findings would also suggest caution in the interpretation of data obtained in the experimental testing of some expanders. From the curves and tables it is clear that in tests of these solutions in healthy males with protein levels between 7.0 and 7.5 grams per cent one would report good infused volume retention of 55 per cent at 6 hours; if the tests with this same

solution were done on groups with protein levels between 6.0 and 7.0 or 5.0 and 6.0 grams per cent one would report fair or poor average expansions of 41 and 27 per cent, respectively.

SUMMARY AND CONCLUSIONS

1. The plasma volume expansion and maintenance obtained with a given volume of 6 per cent dextran is variable from individual to individual.

2. The expansion and maintenance correlate directly with plasma protein concentration; they are poor in subjects with protein levels below 6.0 and excellent with levels above 7.0 grams per cent.

3. The disappearance of the expander (the dextran substance itself) is independent of the movement of fluid into and out of the circulation and is governed only by the physical properties of the molecules and of the pores through which they go.

4. The findings suggest caution in predicting the volume expansion obtainable without a knowledge of the protein level. They also indicate that in comparative studies of certain expanders between individuals or groups the protein level may have to be considered as an important variable in the results.

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THE RENAL CLEARANCE OF PLASMA EXPANDERS*

W. METCALF, L. M. ROUSSELOT, AND F. E. GILBERTSON**

It has been demonstrated that the sharp drop in volume expansion in the first few hours following an infusion of an expander is due to the rapid excretion by the kidneys of the low molecular weight fraction which it contains.[1-3] Volume expansion and maintenance have been shown to be much greater and more prolonged with those expanders whose molecular weight spectrum is skewed to the high side and which, therefore, contain relatively little material of low molecular weight. Conversely, both expansion and maintenance are poor with expanders containing a large fraction of low molecular weight.[4] For more exact characterization of these renal losses and thus better understanding of the behavior of various expanders, renal clearances were determined for the four expanders studied in this laboratory.

METHODS

Under basal conditions groups of 12 essentially normal patients were given an infusion of 500 or 1000 cc of one of the following four expanders: Laros dextran, CSC dextran, modified fluid gelatin, and oxypolygelatin. Venous blood specimens for the determination of plasma expander concentration were taken 10 minutes, and 1, 3, 6, 12, and 24 hours following the infusion The urines were collected for the periods between the blood specimens. The dextran determinations in serum and urine were performed by an alcohol precipitation turbidimetric procedure[5] and the gelatins by a sulfuric acid turbidimetric method[6]

The plasma clearances per hour of the four expanders were calculated according to the standard formula· Clearance (cc.) $= (U \times V)/P$, where U is the urine expander concentration, V the urine volume, and P the mean plasma concentration for the period in which the urine was collected. The values were calculated *per hour* rather than per minute, as is usually done, to avoid fractional volume figures The clearances were calculated in each subject for each of the 5 periods following infusion, 1, 2, 3, 6, and 12 hours The values in the given periods for the subjects in each expander group were then averaged.

RESULTS

The average clearances in the periods following infusion are given in Table 1.

For the various expanders the average clearances in the first hour following infusion ranged between 600 and 1900 cc. per hour. In the last period the values were between 20 and 30 cc. per hour. Although the average drops in plasma concentration from the first to the last period were only twofold for the dextrans and three- to fourfold for the gelatins, the corresponding drops

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in clearance were 20- to 30-fold for the dextrans and 60- to 100-fold for the gelatins.

In comparing the four expanders it may be noted (from the figures in the vertical columns) that the average clearances are inversely related to the mean molecular weights given for these expanders. (The figures for oxypolygelatin beyond 3 to 6 hours do not follow this trend because most of the

Table 1. Average Renal Clearance (cc. per Hour)

EXPANDER	MOLECULAR WEIGHT	PERIODS				
		0–1	1–3	3–6	6–12	12–24
Dextran (Laros)	66,000	634	283	145	51	21
Dextran (CSC)	56,000	620	479	256	83	38
Mod. fl. gelatin	34,000	905	623	301	84	34
Oxypolygelatin	31,000	1892	708	(172)	(64)	(24)

material had been excreted before this time and the urine concentrations thereafter were too low to determine with accuracy.)

By interpolation of our individual clearance values from the data of Brewer,[7] who determined the renal clearances of various molecular weight dextrans, a molecular weight value for each portion of expander excreted was derived. The average of these values is given in Table 2.

Table 2. Average Molecular Weight ($\times 10^{-3}$)

EXPANDER	PERIODS				
	0–1	1–3	3–6	6–12	12–24
Dextran (Laros)	37	45	53	65	74
Dextran (CSC)	37	38	46	59	66
Mod. fl gelatin	33	36	44	56	72
Oxypolygelatin	24	34	50	64	72

The values derived for the various expanders excreted are in the same descending order as the values given for the materials infused. The values also all increase from the first through the last period (horizontal columns) and reach an asymptotic level of about 72,000.

By grouping the individual molecular weight values for each expander into decades, and by adding the amounts of expander represented by each value in the decade, the fractional representation of each decade in the total was calculated The cumulative molecular weight distribution of the materials excreted are given in Table 3.

Twenty-seven per cent of the oxypolygelatin excreted was calculated to

Table 3 Cumulative Molecular Weight Distribution (% by Weight)

	LAROS DEXTRAN	CSC DEXTRAN	MOD. FL. GELATIN	OXYPOLY-GELATIN
10–20,000				27.0
20–30,000	8.9	23.1	37.2	77.5
30–40,000	42.3	46 9	71 9	93.9
40–50,000	69 6	74 6	89.7	95.7
50–60,000	77.1	91.9	96 2	99.4
60–70,000	93.1	99 5	99.3	100 0

have a molecular weight below 20,000. None of the other materials fell in this low range. Almost all of the oxypolygelatin excreted, 93.9 per cent, had a molecular weight of 40,000 or less, whereas 71.9 per cent of the modified fluid gelatin, 46 9 per cent of the CSC dextran, and 42.3 per cent of the Laros dextran was below this level. The corresponding values for the percentages of the material infused having a molecular weight below 40,000 (from the clinical and physicochemical distributions figures) were 73 6, 71.3, 44.5, and 40.2 per cent, respectively.

It should be noted, too, that practically all (93 to 100 per cent) of each of the expanders excreted was calculated to have an average molecular weight of 70,000 or below.

DISCUSSION

The above clearance values can be considered only very approximate because of the methods used and the polydisperse character of the materials tested However, in view of the general agreement of the derived data with the data from other sources, certain tentative conclusions are drawn.

The highest individual clearance noted was 3000 cc. per hour or 50 cc. per minute. The fact that all values were below the generally accepted clearance values for creatinine or inulin, 130 cc. per minute, indicates that these substances are not excreted by the tubules. The low clearance values do not exclude the possibility of tubular reabsorption. However, the relatively complete independence of clearance from plasma concentration makes this unlikely. This implies that the excretion of these expanders is largely, if not completely, a process of filtration through the glomerular capillaries.

Renal clearances are fairly constant over wide ranges of concentration for most substances. The 50- to 100-fold drop in the clearance of these expanders is, therefore, probably not due to the concomitant relatively small 2- to 4-fold drop in their plasma concentrations. It is most likely the result of the rapid shift toward the higher ranges in the molecular weight spectra secondary to the early rapid excretion of the lower molecular weight fractions. The remaining larger molecules diffuse so much more slowly through the glomerular pores that the clearances diminish proportionately.

It is interesting that the limiting molecular weight values obtained from the extrapolation of the clearance data were around 70,000, and, also, that practically 100 per cent of the material excreted was below this molecular weight. These findings conform to the fact established by Homer Smith[8] and others[9] that the limiting size for a substance to be excreted by the kidney corresponds to a molecular weight somewhat below that of albumin, or about 69,000

The actual percentage of expander substance infused which was excreted in 24 hours for the two dextrans, modified fluid gelatin, and oxypolygelatin was 38, 44, 62, and 71 per cent, respectively Thus both the clearances and total excretion of these substances are inversely proportional to their molecular weights. By a process of filtration then, the kidneys eliminate very rapidly from the body that portion of the expander which is of low molecular weight, i.e, below 70,000, and with it an osmotically equivalent volume of plasma fluid. A certain fraction of the low molecular weight material also is "cleared" by the body capillaries into the extravascular space, but it is returned to the circulation by the lymph and thus is also ultimately excreted. Although the low molecular weight expanders have, gram per gram,

a higher osmotic potential than the high molecular weight substances, their more rapid loss through the kidneys results, actually, in a much lower expansion and volume maintenance. On the basis of the foregoing an expander with a narrow molecular weight spectrum concentrated in the region of 70,000 could be expected to have the most effective volume expansion and maintenance properties.

SUMMARY AND CONCLUSIONS

The renal clearances of four plasma expanders have been determined. Both the clearances and total excretion were found to vary inversely with the molecular weights. The latter thus determine the magnitude of the renal losses, and therefore, the efficacy of any expander in volume expansion and maintenance. Certain molecular weight characteristics were also derived from the clearance data and a renal excretion threshold of around 70,000 was found. An expander with a very narrow molecular weight spectrum in this region would probably be the most efficient plasma substitute.

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TOTAL BODY POTASSIUM AS MEASURED BY RADIOACTIVE POTASSIUM (K^{42})*

GEORGE C. HENEGAR, NADINE FOREMAN, GEORGE D. MICHAELS, AND LAURANCE W. KINSELL**

Plasma levels of potassium frequently fail to give a reliable indication of depletion of the total body potassium. This has been found to be true in such conditions as diabetic acidosis, burns, major trauma, Addison's disease, Cushing's syndrome, etc.[1] The plasma potassium represents only a small portion of the total body content of that ion, approximately 5 per cent of the total body potassium. With these facts in mind, a technique for estimation of total body or intracellular potassium ion was sought, utilizing radioactive potassium, K^{42}.

K^{42} has been utilized for this purpose previously, but the results have been equivocal, in part at least because of the use of material of low specific activity, and relatively inefficient instruments.[2-7] However, with the availability of carrier-free potassium-42 chloride solution from the cyclotron at the University of California, and with the development of the deep well scintillation counter by Anger, direct measurement of plasma, red cell sediment, and tissue blocks becomes feasible.[8]

In this study, the K^{42} levels of red blood cells, plasma, and urine were determined.

METHOD

Four hundred microcuries of carrier-free potassium-42 chloride solution was administered intravenously to each patient after the bladder had been emptied Simultaneously, 1 ml of phenolsulfonephthalein solution was given to aid in the estimate of renal function. At intervals of 30 and 120 minutes, respectively, 10 ml. of blood were drawn into tubes containing heparin, and at the same time interval, urine specimens were obtained. A hematocrit reading was made before the plasma was separated from the red blood cells. Plasma samples and red cell sediment samples were counted, the latter after two washings with physiologic saline solution. Two milliliters of plasma and of urine, respectively, were counted in duplicate samples in the deep well scintillation counter. One milliliter of red cell sediment was counted in each of the duplicate samples The red cell readings were then corrected to a 2 ml. volume, in the final calculations. All readings were corrected for decay and background

The phenolsulfonephthalein excretion was determined by a standard technique. On each test day, the plasma potassium was determined by flame photometry.

Studies have so far been carried out in six "normal" individuals, three

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patients with known or probable depletion, and one patient in whom an excess of potassium was administered in the diet. Several studies were carried out in the high-potassium-intake patient and in one purposefully potassium-depleted patient, prior to, during and following the investigative period.

One of the depleted patients was a 62 year old white male, who had been on therapeutic doses of ACTH and hydrocortisone for a period of two weeks with no potassium supplement to his diet. He was clinically hypopotassemic when the isotope studies were carried out. One of the purposefully potassium-depleted patients was maintained on a formula diet which contained less than 50 mg. of potassium chloride daily. The third depleted patient was studied on the metabolic ward. He received a chemically constant

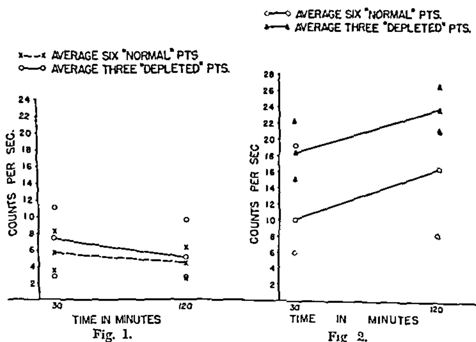


Fig. 1. Fig. 2.

FIG. 1 Rate of disappearance of K^{42} from plasma at 30 and 120 minutes post injection.
FIG. 2. Rate of incorporation of K^{42} into the red blood cell at 30 and 120 minutes post injection.

formula diet which contained 25 mg. of potassium chloride a day for a period of two weeks after the base-line levels were obtained. He was given 5 gm. of sodium chloride, 120 gm. of protein, and 193 gm. of fat, totaling 2200 calories. At the conclusion of the depletion study his formula was supplemented with 10 gm of potassium chloride daily for one week, and a final study was performed at this time.

EXPERIMENTAL FINDINGS

Plasma levels of K^{42} were determined at 30 and 120 minutes, and in each instance showed a lower number of counts per second at the latter time interval than at the ... shows the average of six normal adults. T ... the normal figures for the plasma levels an ... levels of the depleted

individuals. Differences in rate of disappearance of plasma K^{42} between

inute time
its studied.

Table 1. PSP Excretion in Patient DUT

DATE	9/9/54		9/10/54		9/23/54		9/29/54	
TIME (MIN)	VOLUME (CC.)	% EX-CRETED	VOLUME (CC)	% EX-CRETED	VOLUME (CC)	% EX-CRETED	VOLUME (CC.)	% EX-CRETED
30	60	55	120	40	60	15	32	30
120	30	16	50	12	130	5	165	30
Total	90	71	170	52	190	20	197	60

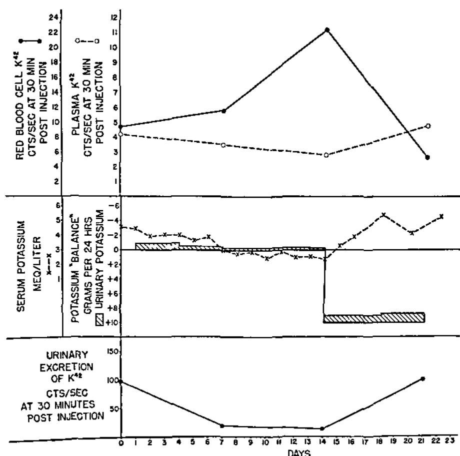


Fig. 3 Serial studies on plasma, red blood cell levels, and urine excretion of K^{42} in a patient studied during dietary potassium depletion In calculating the data on the basis of percentage of administered dose, the curves for rate of incorporation of K^{42} into the red blood cell are similar to those shown but at a lower level.

The range in values at the two time intervals in the red cell studies was not as great as that in the plasma (Fig. 2). The rate of incorporation of K^{42} into red cells in the three depleted individuals was higher than in the "normals."

Patient DUT was studied under quantitatively constant conditions on the metabolic ward. Major potassium depletion resulted in a fall in serum potassium and a progressive increase in red blood cell K^{42} incorporation at both 30 and 120 minute intervals. During potassium depletion, the urinary excretion of the isotope was reduced. His PSP excretion also was reduced at this time (Table 1). Correction of his potassium depletion resulted in a

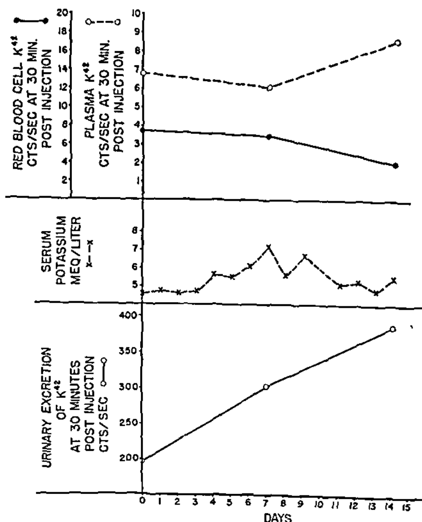


Fig 4 Serial studies on plasma, red blood cell levels, and urine excretion of K^{42} in a diabetic patient on an excess of dietary potassium In calculating the data on the basis of percentage of administered dose, the curves for rate of incorporation of K^{42} into the red blood cell are similar to those shown but at a lower level.

individuals. Differences in rate of disappearance of plasma K^{42} between "normals" and potassium-depleted individuals are not significant.

The red cell uptake figures reveal a rise in uptake in the 120 minute time interval as compared to that at the 30 minute interval in all patients studied.

Table 1. PSP Excretion in Patient DUT

DATE	9/9/54		9/10/54		9/23/54		9/29/54	
TIME (MIN.)	VOLUME (CC)	% EX-CRETED	VOLUME (CC)	% EX-CRETED	VOLUME (CC)	% EX-CRETED	VOLUME (CC)	% EX-CRETED
30	60	55	120	40	60	15	32	30
120	30	16	50	12	130	5	165	30
Total	90	71	170	52	190	20	197	60

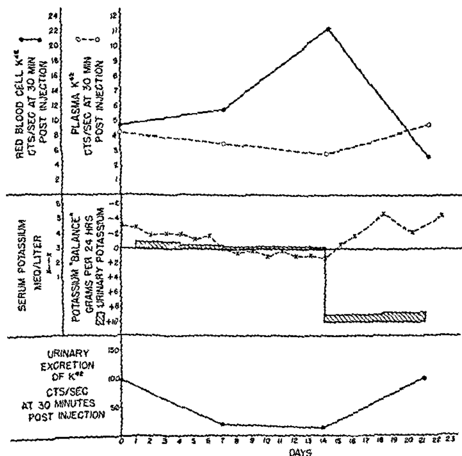


Fig 3. Serial studies on plasma, red blood cell levels, and urine excretion of K^{42} in a patient studied during dietary potassium depletion. In calculating the data on the basis of percentage of administered dose, the curves for rate of incorporation of K^{42} into the red blood cell are similar to those shown but at a lower level.

THE USE OF STABLE RUBIDIUM FOR MEASUREMENT OF TOTAL EXCHANGEABLE BODY POTASSIUM*

JULIAN S. ANSELL AND BERNARD ZIMMERMANN**

Although total body potassium can be measured by using radioactive potassium, the short half-life of this isotope precludes keeping it available for clinical use. In addition, studies with radioactive potassium are difficult to follow more than 48 hours.

The almost identical physiologic behavior of the elements potassium and rubidium[1-3] suggested that the latter stable element might serve as a tracer instead of the radioactive isotope.

This paper presents preliminary work, done in dogs, on the use of stable rubidium as an isotope tracer of potassium in the living mammal.

METHOD

The method in general is that of any tracer dilution study. Pre-injection specimens are drawn, then the tracer substance, in this case rubidium, is administered. Serial samples of body fluids are collected and tracer content is determined for each sample. The values thus obtained are plotted on a graph against time. As the curve of values approaches an asymptote parallel to the time ordinate an arbitrary point is chosen as equilibrium. From this equilibrium value of rubidium, the total exchangeable body potassium is calculated according to the formula:

$$\text{T.E.K.} = \frac{\text{Plasma (K)} \times (\text{injected Rb} - \text{excreted Rb})}{\text{Equilibrium plasma Rb} \times \text{Body Weight}}$$

The maximum safe dose of rubidium for the mammal is approximately 500 mg. per kilogram.[4] To obtain equilibrium plasma levels of rubidium within the range of accurate determination of flame spectrophotometry requires the injection of as little as 50 mg. per kilogram or 10 per cent of maximum safe dose. Our own experience indicates that this dosage is perfectly safe if administered intravenously as a 2 per cent solution at such a rate that the total dose requires 2 minutes or more.

Samples are collected and prepared as follows: Small samples are withdrawn from each vial of rubidium solution prior to injection for concentration checks Samples of blood and other body fluids are drawn just prior to injection and at frequent intervals thereafter until equilibrium is reached. As many samples are drawn thereafter as indicated.

Each blood sample is centrifuged immediately and the supernatant pipetted off and frozen. Hemolyzed samples are discarded. After freezing, the samples are allowed to thaw partly and then mixed with equal portions of 10 per cent trichloracetic acid. When thawing and mixing is complete, doubly distilled water is added to bring the original supernatant to a dilution of one to four. This is centrifuged at 2500 r.p.m. for 10 minutes and the

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** American Scholar in Cancer Research, American Cancer Society.

return of red blood cell K^{42} incorporation to levels below that of the control value (Fig. 3). Similar red blood cell K^{42} findings are noted in the other two depleted individuals (Fig. 2).

Administration of 30 gm. of potassium chloride to patient YOU resulted in initial elevation of plasma potassium, with a subsequent fall to normal. Decreased red blood cell K^{42} incorporation persisted, however, throughout the period of high potassium intake (Fig. 4) Associated with this change, there was an increased urinary K^{42} excretion.

SUMMARY AND CONCLUSION

A method is described for evaluation of radioactive potassium need in the various tissue compartments.

The results thus far show that the rate of disappearance of radioactive potassium from the plasma at the two time intervals (30 and 120 minutes) is not a reliable index of the total potassium needs

The red cell uptake of radioactive potassium may be a good index of the intracellular need, but more data in the normal individual are needed.

Potassium-42, because of its short half-life (12.4 hours), is not a practical clinical test medium, but it serves as a tool in the study of the physiologic activities of this ion

Rubidium-86 may prove to be a more practical clinical agent. Studies with this isotope are being carried out in this laboratory at the present time.

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The error inherent in the flame spectrophotometric determinations with rubidium at these levels is ±1 per cent. Over-all error is estimated at ±2 per cent. This includes errors due to spectrophotometric determinations, pipetting, etc.

RESULTS

In our experiments 6 dogs received injections of 1 gm. of rubidium chloride intravenously as a 2 per cent solution. Curves of plasma rubidium concentration plotted against time fell sharply during the first hour, then levelled off as equilibrium was approached, as illustrated in Figure 1. The six hour concentration was used as equilibrium value. Less than 2 per cent of administered rubidium was excreted in the urine in the twenty-four hours following administration. Average total exchangeable potassium calculated was 41.4 mEq. per kilogram. See Table 1 for results in each animal. This

Table 1.

DOG	WEIGHT KG.	EQUILIBRIUM PLASMA RbCl	PLASMA K^+	T.E.K. MEQ./KG.
33	13 4	7.8	4 8	50 0
209	9.1	11 2	4 8	47.0
238	15 9	6.4	3 8	37.3
245	11.4	8 0	4.8	52.8
268	29.8	5.4	4.7	29.3
269	25 0	5.9	4.7	32.0
			Average	41.4

$$\text{T.E.K.} = \frac{\text{Plasma (K)} \times \text{injected Rb}}{\text{Equilibrium plasma (Rb)} \times \text{body weight}}$$

corresponds closely to values obtained with radioactive potassium by others.[5,6] Range of values: 29.3 –52 8 mEq. per kilogram.

CONCLUSION

We believe that the use of rubidium for the study of potassium metabolism and the measurement of total body potassium by dilution techniques answers the need for a practical, readily available tracer isotope for potassium which can be stored indefinitely and followed over long periods. Studies in the human are in progress.

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supernatant poured into clean tubes for analysis in the flame spectrophotometer. Portions of each sample are set aside for sodium and potassium determinations.

All rubidium analyses in this study were carried out on a Beckman Model DU flame spectrophotometer. Six standard solutions are prepared containing sodium, potassium and trichloracetic acid in the same proportions as the samples to be analyzed In addition, the six standards contain rubidium chloride in concentrations of 10 mg./L., 5 mg./L., 3 mg./L., 2 mg./L, 1 mg./L and 0 mg./L., respectively. One sample from each batch of unknowns is divided into two parts and a known amount of rubidium added

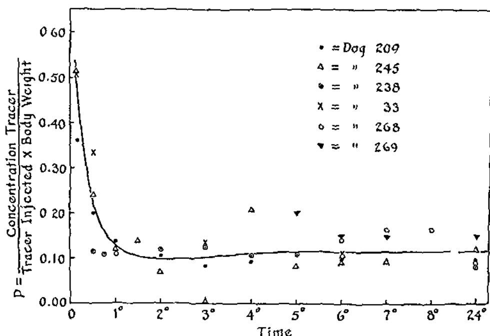


Fig 1 P is the reciprocal of the calculated distribution of administered tracer per unit body weight It is a convenient unit which allows comparison of distribution in animals of different weights

to one part of the sample to act as a "recovery" check on the accuracy of determinations Individual rubidium samples are read at approximately 780 millimicra on the Beckman selector scale. The exact wave length is established by very gently rotating the selector dial for maximum transmittance at a very small slit width. Once this wave length is established, the slit width is opened to 0.1 mm Transmittance readings are then obtained for the appropriate standards, the standard containing 10 mg. rubidium chloride being arbitrarily set at a transmittance reading of 100 with the sensitivity dial.

Dark current readings are checked between every three readings and standards are rechecked every five readings. Curves are drawn by plotting rubidium content against transmittance readings These curves should be straight lines on regular graph paper Unknowns are then read as transmittance scale changes and unknown values estimated by interpolation upon the standard curves.

described below. A suitable vein of the patient was selected and a saline infusion begun. Twenty or 30 ml. of the labelled red cells contained in a calibrated syringe were injected into the rubber tubing of the intravenous set. The injection usually required 45 to 60 seconds. Fifteen minutes later a sample was removed from a contralateral vein of the patient.

The standard was treated as follows: A hematocrit was done on the undiluted red cell suspension. One milliliter was transferred to a 25.0 ml. volumetric flask which was then filled to the mark with 0.007 N NH_4OH. Five milliliters of this 1:25 solution were pipetted into a flat bottom test tube for subsequent measurement of radioactivity. A 1:250 dilution was used for determination of hemoglobin content.

It was then possible to calculate the Cr^{51} content of the labelled red cells in terms of counts per minute per gram of hemoglobin (c.p.m./gm.Hb). The number of grams of hemoglobin in the injected sample could also be calculated, since both the volume and hemoglobin concentration were known.

Samples of blood removed from the patient were treated as follows: About 2 ml. were placed in a test tube containing dried mixed oxalate. The hematocrit and hemoglobin concentration were determined. Five milliliters of the sample were then pipetted into a flat bottom tube and hemolyzed with saponin* for the determination of radioactivity. Total circulating hemoglobin in the patient was calculated as follows:

$$\text{Total Hb (gm.)} = \frac{(\text{c.p.m./gm. Hb in std.})}{(\text{c.p.m./gm. Hb in sample from patient})} \times (\text{gm. Hb injected})$$

Total red cell volume (RCV) was calculated as shown:

$$\text{RCV (ml.)} = 100 \times \frac{\text{Total Hb (gm.)}}{(\text{gm. Hb/100 ml. in sample from patient})} \times (\text{Hct. of sample from patie}$$

Blood volume can be calculated from RCV using established formulae. Corrections for trapping of plasma in the hematocrit may be used in the calculation of RCV if desired.

All measurements of radioactivity were made with a well-type scintillation counter.† The counts were performed so that a counting accuracy of about ±5 per cent or better was achieved. Under the conditions of our experiments a counting rate was obtained with Cr^{51} of about 1×10^5 c.p.m./μc, with a background of about 250 c.p.m.

The procedure used to study hemolytic states was as follows: Blood was removed from a patient, labelled as described above, and reinjected. In some cases the blood was divided into two aliquots, and one of these was given to a subject without hematologic disease. Whenever possible blood from a normal individual was labelled and given to the patient. Samples were taken daily and then at intervals of several days until the half-survival time of the injected cells could be clearly defined.

In order to study post-transfusion survival of erythrocytes from preserved blood the following procedure was used. The preserved blood under investigation was thoroughly mixed in its container and about 30 ml. transferred to a 150 ml. vacuum bottle and labelled with Cr^{51} as described above. A standard was removed after labelling and the blood injected as in the previous paragraphs. Prior to the injection a styleted needle was placed in

* Eastman.

† N. Wood Counter Laboratories, Chicago, Illinois.

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THE USE OF ERYTHROCYTES LABELLED WITH CHROMIUM-51*

TIMOTHY R. TALBOT, JR., MARY F. SAX, ALICE L. CARY, AND ROBERT O. GORSON

It is our purpose in this presentation to describe three practical uses of red blood cells labelled with chromium-51 (Cr^{51})

There have been a number of reports describing the use of Cr^{51}-labelled erythrocytes for the determination of red cell and blood volumes,[1,2] and for studies of red cell survival in vivo, including the evaluation of hemolytic states[1-5] We have made modifications in the methods reported by other workers and will describe the technical aspects which we consider to be improvements

A brief report[5] has already been made from our laboratory describing the use of Cr^{51}-labelled erythrocytes for the determination of post-transfusion survival of preserved blood.

METHODS

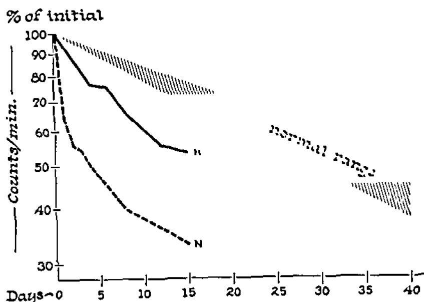
Radioactive chromium was obtained† in the form of sodium chromate. Specific activity has varied from 7 47 to 17 95 millicuries per milligram of chromium.

The determination of the red cell volume of a patient was performed as follows 5 to 6 ml. of neutral citrate or ACD solution were injected into a 150 ml. vacuum bottle.‡ Using a disposable plastic donor set,§ 20 to 50 ml. of blood were removed from the patient into the vacuum bottle. Cr^{51} solution equivalent to about 200 μc was injected into the bottle which was then placed in a refrigerator for 1 to 2 hours at an ambient temperature of 4°C. We have found that constant agitation is not necessary in order to effect a satisfactory uptake of Cr^{51} by the erythrocytes. The bottle was centrifuged for 30 minutes at 1500 r p.m. in an International refrigerated centrifuge at 6°C and the supernatant plasma withdrawn.§ It was then filled with cold sterile saline solution and again centrifuged at 1500 r p.m at 6°C. for 30 minutes The supernatant was removed§ as completely as possible and the remaining cells diluted to approximately their original volume with cold saline. About 5 ml. were transferred to a test tube with a sterile syringe to serve as a standard The treatment of the standard is

* From the Harrison Department of Surgical Research, Schools of Medicine, University adiology, Hospital of the University of Penn- was supported in part by a research grant, al Heart Institute of the National Institutes of

† Abbott Laboratories, North Chicago, Illinois
‡ Baxter "Plasma-Vac" No H-18
§ Using Baxter disposable aspirating sets No. R-32

Fig. 1.



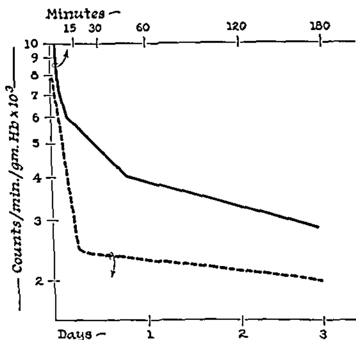


Fig 2.

Fig. 1. Hemolytic anemia demonstrated by short half-survival time of Cr^{51}-labelled erythrocytes. *H* is a patient whose own red cells were labelled and then reinfected. Half-survival time was about 15 days. The disappearance of the same cells in a "normal" is shown in curve *N*.

Fig 2. Post-transfusion survival of erythrocytes from blood which had been stored for 57 days. ... vs the data ... t-transfusic ... the same blood was shown by Ashby's method to have a post-transfusion survival of 22 5 per cent.

a contralateral vein. After injection of the labelled blood, samples were removed at about 1, 3, 5, 10, 20, 30, 45, and 60 minutes, and at about 2, 4, 8, 24, and 48 hours after injection. Radioactivity was then determined in these samples in the usual manner

RESULTS

The use of Cr^{51}-labelled erythrocytes in the determination of red cell volume and blood volume has been documented elsewhere, therefore no further details concerning it will be presented here. The method herein described enabled us to duplicate our results within 5 per cent.

An example of a study in which a diagnosis of hemolytic anemia was confirmed by this method is shown in Figure 1. The patient had a large spleen and questionable hemolytic anemia. It will be observed that the half-survival time of the patient's own red cells reinjected into her circulation was 15 days. The normal range is about 28 to 40 days. In addition the patient's red cells also had a short half-survival time after injection into a patient without hematologic disease.

Figure 2 shows a plot of data from a representative study of the post-transfusion survival of erythrocytes from preserved blood. The curve has at least three components which we have interpreted as follows. (*a*) mixing time, ending at about 5 minutes, (*b*) a "rapid" phase lasting about 30 minutes; and (*c*) a "slow" phase which lasts from 1 to 8 hours depending on the state of preservation of the blood. Thereafter the rate of destruction becomes identical to that of normal fresh blood.

The difference between the specific activity of hemoglobin at the end of mixing time and at the end of the "slow" phase has been shown to be a valid measure of the non-viable portion of preserved blood after transfusion. This was confirmed in 24 experiments designed to compare the post-transfusion survival of aliquots of preserved blood A 450 ml aliquot was studied by means of the Ashby method of differential agglutination, and a 20 to 40 ml. aliquot by means of the method described above. The preserved bloods were from 7 to 57 days old and were shown to have a range of post-transfusion survival of 96 to 23 per cent. The results obtained by these two methods agreed within 6 per cent, except in two obviously unsatisfactory experiments.

DISCUSSION

It is our belief that the determination of total circulating hemoglobin offers certain advantages over a measurement of red cell volume or plasma volume.

There are many questions as yet unanswered concerning the use of Cr^{51}-labelled red cells as a means for studying hemolytic states. The method is often useful in arriving at a diagnosis or in determining the degree of a hemolytic process We believe that the behavior of normal cells in normal individuals requires further study before the slight or "borderline" hemolytic states can be diagnosed with this technique.

Gibson[6] has proposed that after transfusion of preserved blood, the rate of disappearance of the non-viable cells from the recipient's circulation might be used as a measure of the degree of preservation of the blood. Since he was forced to use relatively large amounts of blood, at least 35 minutes elapsed before the first post-transfusion sample was obtained. Thus it was

including the position of the patient on the operating table, the amount and type of preoperative medication and of anesthetic agent, the duration and depth of anesthesia, the effectiveness of the assistance to respiration, and the capability of the anesthetist. In the following clinical study these variables were eliminated wherever possible in an effort better to compare the effectiveness in reducing respiratory acidosis of manual versus machine ventilation.

METHODS

The data in this clinical study are derived from a study of seventy patients undergoing prolonged operations with an open thorax. The operations included fourteen pneumonectomies, thirty-three lobectomies, and twenty-three miscellaneous chest procedures involving the open thorax. Arterial blood samples were analyzed for oxygen content, carbon dioxide content, and *p*H. These samples were drawn at the following intervals: (1) before the administration of the anesthetic; (2) on opening the pleura–average time 1 hour 10 minutes after (1); (3) on closure of the pleura–average time 3 hours after (1); and (4) at the end of the operative procedure–average time 3 hours 30 minutes after (1). Preoperative and postoperative arterial lactic acid and potassium determinations were also made. Not all samples were obtained in every patient.

Blood samples were heparinized and anaerobically collected from the femoral artery and by a Cournand needle inserted in the radial artery. The carbon dioxide content of the plasma was measured using the volumetric method of Van Slyke and Cullen.[2] The partial pressure of carbon dioxide was then read from the line chart of Van Slyke and Sendroy,[3] using the calculated value of the total plasma carbon dioxide in volumes per cent and the observed *p*H. The *p*H was measured with a Model G glass electrode calibrated with buffer solution before each determination. The oxygen content and capacity were determined by the spectrophotometric method of Hickam and Frayser.[4] The lactic acid was determined by the photoelectric colorimeter method of Barker and Summerson.[5] The serum potassium was determined by the flame photometer, the Beckman Model DU.

Anesthesia. Premedication consisted of pentobarbital sodium, gr. 1½, the night before surgery and an equal amount one and one-half hour preceding operation, and morphine sulfate, gr. ⅙, and atropine, gr. 1/150, thirty minutes preceding surgery. Sixty-seven of the patients were induced with Pentothal Sodium, and anesthesia was effected with ether and oxygen administered in a closed re-breathing circuit. With the operating table broken 15 degrees the patients were placed face down; a small sandbag placed longitudinally was used to further elevate the operated side. All operations were performed by one surgeon assisted, in the main, by the same surgical team employing a constant technique and depth of anesthesia. Most of the anesthesia was administered by two anesthesiologists, the remainder being given by two nurse anesthetists.

The seventy consecutive cases were studied in the following four categories: (1) ten cases–hand-bag-squeeze controlled anesthesia, as commonly employed in "open chest" cases; (2) ten cases–hand-bag-squeeze controlled ventilation in which the anesthesiologist made a maximum effort throughout the entire anesthetic period to prevent hypercapnia; (3) forty-one cases –mechanical respiration started at the time of opening of the pleura and

impossible to measure the initial phase of destruction, and a direct measurement was not possible.

We believe that our method offers the advantage of direct determination of the entire phase of destruction. In addition, only 20 to 40 ml. of blood are required, and the total time needed to complete a single study is considerably less than with any method previously described.

SUMMARY

Three practical and useful applications have been briefly described concerning the use of erythrocytes labelled with chromium-51. These are:

1. The determination of total circulating hemoglobin
2. The evaluation and diagnosis of hemolytic states.
3. The determination of the post-transfusion survival of erythrocytes from stored blood.

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MECHANICAL ELIMINATION OF RESPIRATORY ACIDOSIS DURING OPEN THORACIC PROCEDURES*

WILLIAM H. FALOR, THOMAS R. KELLY, AND CHARLES W. REYNOLDS**

Beecher's[1] demonstration of the development of severe progressive respiratory acidosis during open thoracic procedures has been widely confirmed. Manifested by an increase in arterial carbon dioxide tension and a decrease in arterial pH, this acidosis is of significant and immediate concern to every surgeon utilizing a thoraco-abdominal or thoracic incision. The degree of the derangement in acid-base balance depends upon a number of variables,

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** The authors are indebted for technical assistance to A. Kerkian, S.B., Chief of Clinical Laboratories, and his associates in the Department of Pathology and Laboratories of The City Hospital of Akron.

situated on top of the machine. In this series the peak pressure of the inspiratory phase ranged from 6 to 8 mm. Hg with a respiratory rate of from fourteen to eighteen per minute. The shape of the pressure curve (Fig. 2) is demonstrated and includes a gradual increase in pressure to a plateau maintained for one-fourth the respiratory cycle time. This is followed by a rather rapid decrease in pressure and by a latent phase persisting for approximately one-half the duration of the respiratory cycle with a pressure of 0. This pressure-time relationship would appear to satisfy the requirements established by Motley.[6]

RESULTS

A compendium of the results of the arterial blood determinations from the seventy thoracotomies appears in Table 1. Space limitation prevents inclusion of the supporting data; these will be published in detail at the time of completion of the present study. Table 1 identifies the four categories of cases together with the *p*H and partial pressure of carbon dioxide levels at four periods of the operative procedure. In addition the difference in preoperative and postoperative values is indicated.

Anesthesia for the first ten thoracotomies was controlled by the hand-bag-squeeze method, the anesthetist employing the rate and depth of ventilation deemed by him to be satisfactory to maintain the patient between the second and third plane of the third stage of anesthesia. In this group of six lobectomies and four miscellaneous thoracotomies, as in all subsequent groups, the miscellaneous procedures often involved poor risk patients and major esophageal surgery and accounted for a number of the highest carbon dioxide "pile-ups." Of the ten patients, nine showed in at least one of the four arterial samples a carbon dioxide tension above 50 mm. Hg, the accepted upper limit of normal. The postoperative average rise of 11.5 mm. Hg thus does not reflect the actual curve of carbon dioxide values. It should be noted that much of the "pile-up" occurred prior to pleurotomy and was complemented during the "open" thoracotomy, and was partially reduced by the end of the operation.

The second category of ten thoracotomies includes four pneumonectomies, five lobectomies and one miscellaneous procedure; six of these patients were particularly poor risks. In this group the anesthesiologist made a calculated attempt during the entire anesthesia by the simple bag-squeeze method to prevent carbon dioxide retention. In seven of the ten cases the carbon dioxide was at some time above 50 mm. Hg; in only one of the four pneumonectomies did the carbon dioxide tension fail to rise above 50 mm. Hg. During the "open" thoracotomy the degree of hypercapnia was the most marked of the entire series; however, at closure most of this had been dissipated.

In the category of forty-one thoracotomies the Rand-Wolfe breathing machine was used from the time of pleurotomy until pleural closure and occasionally until the end of the operation. In nine of these cases the four arterial samples were analyzed, each case showed at least one carbon dioxide tension of above 50 mm. Hg. Again, the major increase in carbon dioxide occurred prior to thoracotomy, however, during the "open" operation the hypercapnia decreased slightly, and upon completion of the surgery the carbon dioxide pressure was normal.

In the final category of nine thoracotomies there were two pneumonec-

continued at least until closure of the pleura, and (4) nine cases—mechanical respiration started immediately after intubation.

Mechanical respiration was obtained by use of the Rand-Wolfe* mechanical respirator (Fig. 1). Positive pressure, registered in millimeters of mercury pressure on a dial on the upper face of the machine, is obtained by

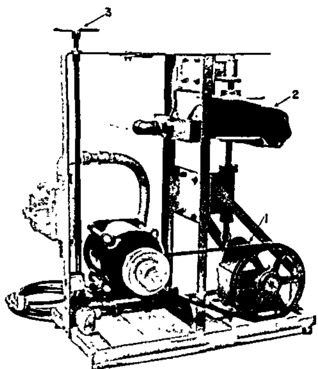


Fig. 1 Internal mechanism of Rand-Wolfe breathing machine. *1*, Cam; *2*, rubber anesthesia bag, *3*, respiratory rate regulator.

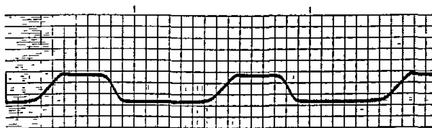


Fig 2. Pressure-time curve of Rand-Wolfe breathing machine using 10 mm Hg pressure at 21 respirations per minute.

compression of a breathing bag (Fig. 1, No 2) between two disks The lower disk *is elevated by a piston operating off of an eccentric cam (Fig 1,* No. *1*) driven by a one-sixth horsepower motor The breathing bag is attached by airtight connections to the carbon dioxide absorber. The positive pressure and respiratory rate are controlled by the volume of anesthetic mixture allowed to enter the breathing bag and by a dial (Fig. 1, No. 3)

* Manufactured by H. J Rand Foundation, Cleveland, Ohio.

tomies, three lobectomies, and four miscellaneous procedures. The mechanical ventilator in this group was utilized from the time of intubation until the patient was removed from the operating table. It should be noted that the large initial increase in carbon dioxide of the original sixty-one cases did not occur in this group. In two of these cases the carbon dioxide tension rose above 50 mm. Hg. (50.5 and 59.5); in one of these cases the original tension was 51 mm. Hg. There was no other significant *p*H or pCO_2 change in any patient in this group at any time.

Arterial serum potassium and lactic acid determinations were performed in thirty of the above patients to establish the relationship of each to the acid-base changes. The lactic acid level remained fairly constant: the potas-

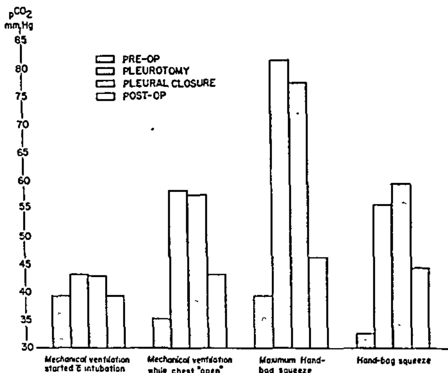


Fig. 3. Graphic comparison of carbon dioxide pressures during thoracotomy using manual or mechanical controlled anesthesia.

sium was elevated in all categories, being highest in the manually controlled —average rise 5.21 to 5.93 mg. per cent.

DISCUSSION

In a series of seventy consecutive thoracotomies performed in a modified face-down position and employing, in the main, the same surgical team and small group of anesthetists, a comparison of manual versus machine controlled ventilation has been attempted. A comparison of the effectiveness in this series of manual versus machine methods for reduction of carbon dioxide "pile-up" appears in Figure 3.

The greatest sustained increase in carbon dioxide was observed in the group given controlled ventilation. The large group controlled after pleurotomy by the mechanical ventilator and the group given maximum assistance by the anesthesiologist showed comparable ultimate carbon dioxide

Table 1. Categories of Study of Respiratory Acidosis Occurring during Manual and Machine Controlled Ventilation

	PRE-OP		PLEUROTOMY		PLEURAL CLOSURE		POST-OP		CHANGE PRE- TO POST-OP	
	pH	pCO_2	pH	pCO_2	pH	pCO_2	pH	pCO_2	pH	pCO_2
Hand-bag-squeeze 10 Thoracotomies (6 lobectomies, 4 miscellaneous)	7.40	32.6	7.27	55.3	7.20	59.0	7.33	44.1	−0.07	+11.5
Maximum effort to prevent hypercapnia 10 Thoracotomies (4 pneumonectomies, 5 lobectomies, 1 miscellaneous)	7.35	39.4	7.10	81.0	7.10	77.0	7.30	46.2	−0.05	+6.8
Mechanical ventilation during "open" thoracotomy 41 Thoracotomies (8 pneumonectomies, 19 lobectomies, 14 miscellaneous)	7.43	35.8	7.24	58.0	7.21	57.0	7.35	43.2	−0.08	+7.4
Mechanical ventilation started on intubation 9 Thoracotomies (2 pneumonectomies, 3 lobectomies, 4 miscellaneous)	7.40	39.3	7.40	43.5	7.36	43.3	7.36	39.6	−0.01	+0.3

THE USE OF ALBUMIN I^{131} (RIHSA) FOR PROTEOLYTIC AND ANTIPROTEOLYTIC ACTIVITY DETERMINATION*

KAREL B. ABSOLON**

An adequate and technically simple method for proteolytic and antiproteolytic activity determination is of interest to any laboratory where studies concerning the function of the stomach, pancreas and antitryptic factors are performed. Numerous methods have been devised for these purposes. These methods include chemical, physical, photometric analyses[1-4] and electrometric methods.[5] During the studies concerning the fate of intravenously administered radioactive iodinated human serum albumin (RIHSA), part of the radioactive material was recovered in the gastric juice. Examination of this portion by filtration through a semipermeable membrane showed that the isotope no longer was bound as protein-I^{131} complex. One possible explanation was the splitting by the gastric content, which suggested that the amount of split fractions from RIHSA could be used as an index of proteolytic activity. In this study the activity of the gastric juice and feces has been compared with the activity of crystalline pepsin and trypsin; in addition, this method was given a trial with limited clinical material.

PRINCIPLE

The basic technique is one of incubating the RIHSA with the test solution and then separating the diffusible I^{131} portion from the protein. The enzymatic activity corresponds to the magnitude of the radioactive split products. This separation was first accomplished by filtration of the material through cellophane. In later experiments an alternate method was used, namely the separation of the two fractions by centrifugation after precipitating the undigested radioactive protein at the end of the incubation period. The two procedures give similar readings under identical circumstances.

MATERIALS

The substrate was a tracer amount of RIHSA† with a carrier protein diluted in a citric acid and disodium phosphate buffer of pH 7.8 for trypsin and 2.2 for pepsin to give in the final concentration a count of approximately 200,000/0.5 ml./min. Although no carrier is needed if the separation of the split radioactive fraction is accomplished by filtration, one is essential if precipitation is used.

Egg albumin, ox hemoglobin and casein were found satisfactory as carriers. Because egg albumin exhibited more antitryptic activity than could

* From the Department of Surgery, University of Minnesota Hospitals, Minneapolis. This study was supported by funds from the following sources: (1) Malignant Disease Research Fund, (2) Jay and Rose Phillips Fund, (3) Watson P. Davidson Fund, (4) Austen S. and Anne Cargill, (5) R. C. Lilly Fund.

** The author wishes to Department of Biochemistry, University of echnique which improved this test to Dr. O , Dr. J. Marvin, Department of Radiology, and Dr. E. Benson, Department of Laboratory Medicine, for advice, and to Dr. J. Fusaro, Mr. R. Yoder and Miss Betty Frazee for technical assistance.

† Abbott, 10 mg. per milliliter.

retention. Several of the patients, however, in the maximum control group were poor risks and rapidly developed a profound degree of respiratory acidosis, they thus markedly raised the average values of the entire group. Acidosis, once established, could not be reduced by the machine; however, acidosis never developed in that group of patients in whom the mechanical ventilator was started at the same time that the patient was intubated.

Common to all groups is a rise in carbon dioxide during the period prior to pleurotomy. This may be the result of respiratory depression secondary to Pentothal Sodium induction but is undoubtedly related as well to the modified face-down position employed.

In thirty of the fifty mechanical ventilation thoracotomies there was no appreciable change in the *p*H or pCO_2 In nine of this group the use of the Rand-Wolfe breathing machine from the time of intubation eliminated respiratory acidosis.

CONCLUSION

In this series of seventy "open" thoracotomies the Rand-Wolfe breathing machine has been more effective than manual methods in the prevention of respiratory acidosis This study is still in progress.

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increased, the percentage splitting increasing slightly (Fig. 1). The non-radioactive portion of the substrate exhibited a limiting factor; a decrease of the protein carrier caused a marked increase of the radioactive split products and percentage activity (Fig. 2).

The sensitivity of the determination can thus be varied as needed for determination of minute amounts of enzyme, by taking into account also the optimum temperature and time of incubation. The latter was in direct relationship to the amount of splitting. As the albuminolysis occurred rather rapidly, a relatively short incubation period of one hour was considered adequate for most experiments (Fig. 3). The enzymatic activity of pepsin

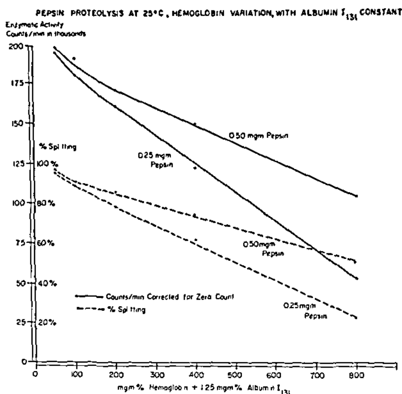


Fig 2.

and trypsin was depressed by lowering the temperature to 10°C. and inhibited by high temperatures, the optimum being 37°C. (Fig. 4).

An increase of temperature split small amounts of I^{131} fractions from the substrate proportionally up to 56°C.; at 66°C. coagulation occurred and the split portion decreased. The enzymatic reaction was inhibited in proportion to amount of added trichloracetic and sulfosalicylic acid. The influence of variation of pH was one of inhibiting the activity of pepsin at a high pH and of trypsin at a low pH. There was no influence of the pH on the substrate. In a solution of pepsin and trypsin the two enzymes could be differentiated by varying the pH. For quantitative calculation of an unknown specimen the count is compared (read from graph) to the activity (count) of known dilutions of the enzyme, taking into account the constant percentage of free I^{131} of the substrate, or expressed as percentage splitting.

be explained by competitive inhibition, hemoglobin was the choice. In peptic proteolysis no antienzymatic effect could be observed and the proteolysis with albumin and hemoglobin as carriers was identical. Fifty to 200 mg. per cent of the hemoglobin was found satisfactory for pepsin and trypsin proteolysis and adopted in this laboratory for future studies.

The standard solutions of enzyme were made from Pepsin USP and Tryptar (Armour). The gastric juice was aspirated via an indwelling Wangensteen tube and filtered Fresh feces were obtained on the ward, diluted in a buffer of pH 7.8 and, after emulsification, centrifuged; the

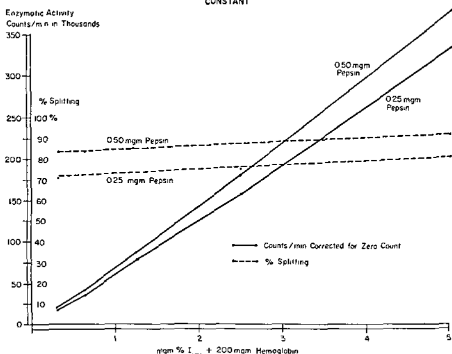


Fig. 1.

supernatant and its dilutions became the unknown All counts were made using a well scintillation counter

PROCEDURE

Two milliliters of substrate was put into each serologic tube. One milliliter of a known amount of enzyme or unknown material was added and mixed. The incubation period was one hour at 37°C. The precipitation of undigested protein was accomplished with 15 per cent sulfosalicylic acid, 0.5 ml. of supernatant was carefully pipetted off for counting

RESULTS

Increasing the concentration of enzyme resulted in a proportional increase of split products up to a certain point, further increase was in direct relationship to the amount of radioactive protein carrier. Greater dilutions of enzyme caused relatively more splitting when the amount of RIHSA was

By varying the pH when a mixture of pepsin and trypsin was used, similar peaks, as in the case of the individual enzymes, could be demonstrated. It may be true that at a certain pH mutual inhibition occurred and no summation of proteolysis, as might have been expected. It was the purpose of this study to demonstrate the relationship of different factors as they are known to influence enzymatic reactions to proteolysis of RIHSA; the amounts of radioactive substrate varied from one to the other experiment. If the conditions of the experiment were kept constant an identical degree of splitting was obtained.

CLINICAL APPLICATION

The preceding studies have shown that by using the described technique, trypsin and pepsin can be determined accurately and simply. The clinical

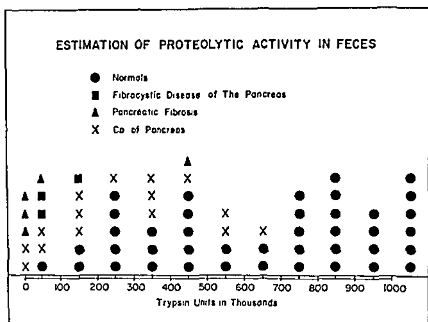


Fig. 5.

application was of interest for studies of gastric and pancreatic activity. It would be more convenient to determine the tryptic activity in the feces as an expression of external pancreatic function, as it is easier to determine uropepsin as an expression of gastric peptic activity.

Feces contains protein-splitting enzymes, probably from three main sources: the pancreatic trypsin, proteolytic enzymes of the small intestine and the proteolytic activity of bacteria. It could be therefore expected that even in a case of total pancreatectomy some proteolytic activity would be present in the feces, though the bacterial part might be decreased with use of antibiotics.[9]

Forty-two normal specimens of feces obtained from children and adults were examined and all but three split the radioactive substrate in normal proportions. These three patients had diarrhea or severe constipation, feces must be calculated to the dried weight to obtain correct values, or in the latter case a cathartic has to be administered. The feces of patients with

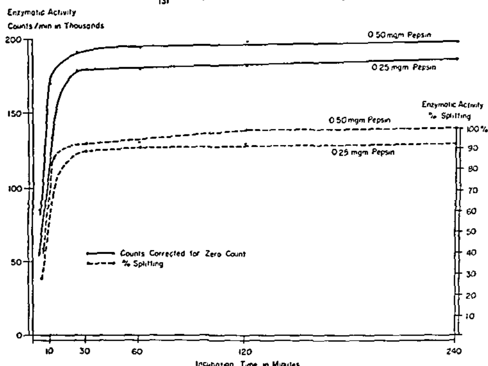

PEPSIN PROTEOLYSIS AT 25°C, VARIATION OF INCUBATION PERIOD, WITH SUBSTRATE ALBUMIN I131 (1.25 mgm %) PLUS HEMOGLOBIN 100 mgm %
Enzymatic Activity
Counts/min in Thousands
0.50 mgm Pepsin
0.25 mgm Pepsin
Enzymatic Activity
% Splitting
0.50 mgm Pepsin
0.25 mgm Pepsin
Counts Corrected for Zero Count
% Splitting
Incubation Time in Minutes

Fig 3.

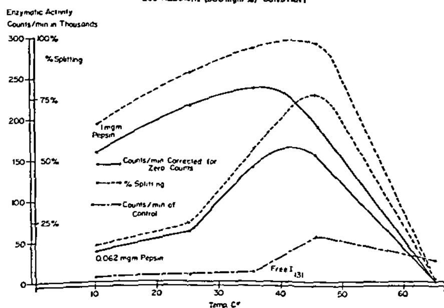

PEPSIN PROTEOLYSIS TEMPERATURE VARIATION, SUBSTRATE ALBUMIN I131 (4 mgm %), + EGG ALBUMIN (500 mgm %) CONSTANT
Enzymatic Activity
Counts/min in Thousands
% Splitting
1 mgm Pepsin
Counts/min Corrected for Zero Counts
% Splitting
Counts/min of Control
0.062 mgm Pepsin
Free I131
Temp. C°

Fig. 4.

PLASMA PROTEOLYTIC ACTIVITY ASSOCIATED WITH EXPERIMENTAL TRANSFUSION REACTION IN DOGS*

A. D. MASON, JR., C. BARBER MUELLER, AND D. G. STOUT

In attempting analysis of the diverse clinical and experimental information which has accumulated concerning the pathogenesis of lower nephron nephrosis, we have become interested in the possible relationship between acute activation of the plasma proteolytic enzymes and this process. Two lines of investigation have led to this interest:

1. Normal mongrel dogs given initial infusions of human blood develop hemoglobinemia and hemoglobinuria without evidence of renal impairment. Animals sensitized to human blood, on the other hand, have a 25 to 30 per cent incidence of renal failure when given similar infusions.[1] Since hemoglobinemia per se, hypotension per se, and combinations of these appear ineffective in consistently producing experimental renal disease,[2-4] it would appear that some other component of this anaphylactoid reaction to blood bears an etiologic relationship to the disease. One such component is increased proteolytic activity in plasma.[5, 6]

2. Reported cases of high "spontaneous" plasma proteolytic activity in previously normal human beings show a striking correlation with the occurrence of tissue injury and traumatic shock.[5] Since such insults are prone to produce acute renal failure, we infer a possible correlation of such proteolytic activity with the occurrence of subsequent renal disease. The probability of such activity in human hemolytic transfusion reactions is high, but has not, to our knowledge, been proven.

This is a report of our initial observations in an investigation of this relationship. We have chosen to measure plasma fibrinolysin activity in vivo as an index of the degree of plasma proteolytic activity in a group of dogs receiving their first and second infusions of human blood.[1]

PROCEDURES

Adult female mongrel dogs used throughout this investigation were maintained on standard laboratory rations without water restriction. During an experiment they were gently restrained on a small table without anesthesia. The animals were divided into two groups, group I receiving human blood for the first time and group II receiving human blood for the second time after a 3 to 4 week interval. We have previously reported data from a series of animals treated in this fashion in regard to renal function as determined by PAH and inulin clearances and subsequent analysis of blood urea nitrogen. In group I none of the animals became ill, none died and none showed any evidence of impaired renal function after the administration of 10 to 25 ml of human blood per kilogram. In group II no animal survived the administration of more than 5 ml. of human blood per kilogram and about 30 per cent of these animals experienced a period of acute renal

* From the Department of Surgery, Washington University School of Medicine, St. Louis, Missouri. This work aided by a grant from the U. S. Public Health Service. A-96 (C2R).

pancreatic insufficiency shows a decrease or absence of albuminolytic activity

Four patients with confirmed carcinomas of the pancreas, three patients with pancreatic insufficiency due to fibrocystic disease, one with diabetes and calcification and two diabetics with chronic relapsing pancreatitis could be identified as having a defect in digestion by this method (Fig. 5). An increase in fecal tryptic activity was demonstrated in individuals with pancreatic insufficiency after oral medication with Pancreatin USP.

When human serum was added in increasing amounts to the substrate, containing equal proportions of trypsin, the proteolytic reaction was inhibited by adding an antitryptic factor and/or by increasing the protein content of the substrate. For determination of gastric peptic activity and uropepsin a similar method was successfully used.

DISCUSSION

The commonly used simple liquefaction of gelatin and film test techniques for tryptic activity has certain undesirable features[6, 7] such as lack of accuracy and reliability Radioactive tracer methods in enzyme determination present many advantages, i e., Geiger counting will give an accurate numerical evaluation independently of the color of the examined specimen, which, using other methods, presents considerable difficulties.[5] In the samples incubated with identical amounts of enzyme, the I^{131} liberated had a mean count of 388,100/min. (S D. 842/min). This S D. approximated that to be expected from the observed Geiger counter error of 0 2 per cent at the same activity level The accuracy of this technique is easily apparent from such a calculation, i e, when the activity of sample A and sample C is determined and compared to unknown B, which is a mixture of ½ A plus ½ C; a theoretical calculation derived from adding ½ of the count of A and C should equal the activity of unknown sample B. The errors prove to be less than 1 per cent by this method

SUMMARY AND CONCLUSIONS

An enzymatic test for in vitro determination of peptic and tryptic activity has been developed using RIHSA as substrate, it allows accurate estimations of enzymatic activity The simplicity allows this technique to be used for detection of clinical cases of enzyme deficiencies in question. It remains to be found how close a relationship exists between pancreatic function and the fecal tryptic activity This method was applied to a few clinical cases of rather marked enzymatic aberrations and proved to have some potential diagnostic value.

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Table 1. *Activity of Dog Plasma after First Infusion of Human Blood**

EXPER NO DOG DATE	MINUTES AFTER INFUSION	FIBRINOGEN 0 3 ML	FIBRINOGEN 0 15 ML.	FIBRINOGEN 0 075 ML.	CASEIN CASEIN UNITS	WHOLE BLOOD CLOT MINUTES	REMARKS
FV-9	Pre	+	+	+		X	Urine output in 5 min.
124-H	10	++	++	++	X	X	
2-10-54	60	++	++	++		X	
FV-10	Pre	+	+	+		5	Urine output in 25 min.
126-H	10	+	+	++	X	5	
2-17-54	60	+	+	+		5	
FV-11	Pre	++	+	++		3	Urine output returned in 30 min.
125-H	10	++	++	++	X	20	
2-16-54	60	++	++	++		20	
FV-13	Pre	0	0	+	16	5	Urine output returned in 30 min
133-H	10	++	++	++	14	6	
6-8-54	60	++	++	++	23	5	
FV-14	Pre	++	++	++	5	4	Urine output in 50 min.
134-H	10	+	++	++	16	75	
6-10-54	60	++	++	++	15	15	
FV-15	Pre	++	++	++	4	9	Urine output in 30 min.
135-H	10	+	+	++	10	10	
6-15-54	60	++	+	++	9	X	
FV-16	Pre	++	++	++	25	4	Urine output in 10 min.
136-H	10	++	++	++	25	X	
6-17-54	60	++	++	++	28	4	
FV-17	Pre	++	++	++	6	6	Pregnant dog, aborted after first human blood
137-H	10	++	++	++	18	6	
6-22-54	60	++	++	++	17	X	

*All of these animals received 10 ml of human blood intravenously. None died and none developed evidence of acute renal disease.

impairment lasting from three days to three months. The series of experiments reported here attempts to evaluate differences in the proteolytic activity of plasma immediately following the first and second infusion.

Human blood obtained from the Barnes Hospital Blood Bank was infused into each animal at a rate of 5 ml. per minute. Samples of blood were drawn from the dog prior to the infusion, and 10 minutes and 60 minutes following the cessation of infusion by way of an intravenous polyethylene catheter. An indwelling catheter was inserted into the bladder and urine samples were obtained pre-infusion and 60 minutes post-infusion in all experiments in which urinary excretion was restored after 60 minutes.

Estimations of the proteolytic activity of plasma were carried out as follows:

Whole Blood Clotting Time. One milliliter of whole blood was placed in a plastic test tube as soon as drawn and the time for the formation of an invertible clot recorded. This time is expressed in minutes and anything under 10 minutes is regarded as normal. Times over 2 hours are reported as infinite clotting time.

Plasma Proteolytic Activity. Nine parts of whole blood were mixed with one part of a 3.2 per cent solution of sodium citrate as soon as drawn. This blood was centrifuged immediately, the plasma removed and mixed with 0.032 per cent acetic acid in proportions of 1:19 The euglobulin fraction of plasma precipitated by this procedure was separated by centrifugation and redissolved in phosphate-saline buffer at pH 7.4. The volume of buffer was equal to the original volume of plasma. This crude fraction contains fibrinolysin and its precursor, profibrinolysin [7]

Fibrinogen Digestion Test of Plasma. To 0 5 ml samples of 0 4 per cent solution of bovine fibrinogen (Armour*) made up in phosphate-saline buffer, pH 7.4, are added 0.3 ml, 0 15 ml, and 0.075 ml. aliquots of euglobulin solution Each is diluted to 0.8 ml with phosphate-saline buffer and incubated at 37°C for 30 minutes. 0.2 ml. of thrombin (Parke-Davis), 100 u/ml in phosphate-saline buffer, is then added, mixed with gentle shaking and the tubes are incubated for another 30 minutes. At this time the tubes are read as follows. ++ = invertible clot, + = solid clot which fills the tube but does not adhere to the sides, ± = small clot floating in solution, 0 = no discernible clot. A ++ or + value in the tube containing 0.3 ml. of euglobulin solution is considered normal in the dog.

Casein Digestion Test of Plasma Casein is prepared from skim milk by triple precipitation and alcohol ether extraction and adjusted to pH 7.4. To 5 ml. of 5 per cent casein, 4 ml. of phosphate-saline buffer and 1 ml of euglobulin solution is added This mixture is incubated at 37°C. for 18 hours. 2 ml. samples are withdrawn at one minute and at 18 hours after
4 ml of 5 per cent trichloracetic
laced in a Coleman cuvette. To
ml. of Folin-Ciocalteau reagent
(Fisher No. So-P-24) which has been diluted with twice its volume of distilled water. The cuvettes are left at room temperature for one hour and optical density is then recorded at 660 μ in a Coleman Junior Colorimeter. This test measures the amount of tyrosine and "tyrosine-like" substances released from casein by the proteolytic activity of the euglobulin solution.

* Supplied through the courtesy of A H Holland, Armour Laboratories, North Chicago, Illinois.

FV-10	Pre	0	+	+		5	35 ml A+
126-II	10	0	0	0	X	9	1st inf. 2-17-54
3-9-54	60	0	0	±		15	BUN elevated 3-10-54
FV-11	Pre	0	0	±		5	35 ml. A+
125-II	10	0	0	0	X	∞	1st inf. 2-16-54
3-11-54	60	0	±	+		∞	BUN elevated 3-19-54
FV-12	Pre	+	++	++		8	30 ml. A+
126-II	10	+	++	++	X	∞	1st inf. 2-17-54
3-21-54	60	++	++	++		∞	Died 1 hr. post-inf
FV-18	Pre	++	++	++	20	2	36 ml. 0+
133-II	10	+	+	++	30	∞	1st inf. 6-8-54
7-6-54	60	++	++	++	6	∞	Died 6 hr. post-inf.
FV-19	Pre	+	++	++	20	2	
135-II	10	+	++	++	35	2	1st inf. 6-15-54
7-8-54	60	++	++	++	19	1	Dog well 7-9-54
FV-20	Pre	++	++	++	17	1	45 ml 0+
136-II	10	+	++	++	34	∞	1st inf. 6-17-54
7-12-54	60	++	++	++	20	3	Died 1 hr. post-inf.
FV-21	Pre	++	++	++	10	2	10 ml.
134-II	10	++	++	++	17	2	1st inf 6-10-54
7-14-54	60	++	++	++	16	2	Dog well 7-15-54
FV-22	Pre	±	+	+	47	3	
137-II	10	+	+	+	57	∞	1st inf. 6-22-54
7-22-54	60	++	++	++	21	∞	Died 1 hr. post-inf.

*No animal received more than 45 ml. of blood. All were extremely ill and had the usual signs of clinical shock.

*Table 2. Activity of Dog Plasma after Second Infusion of Human Blood**

EXPER NO DOG DATE	MINUTES AFTER INFUSION	FIBRINOGEN 0 3 ML	FIBRINOGEN 0 15 ML	FIBRINOGEN 0 075 ML	CASEIN CASEIN UNITS	WHOLE BLOOD CLOT MINUTES	REMARKS
FV-1	Pre	+	++	++		3	50 ml A+
120-H	10	++	++	++	X	X	1st inf 12-11-53
1-19-54	60	++	++	++		X	Died 1-19-54
FV-2	Pre	0	0	+		X	35 ml A+
121-H	10	0	0	+	X	5	1st inf 12-15-53
1-21-54	60	0	0	+		5	10 min clot lysed in 1 hr.
							BUN elevated 1-22-54
FV-3	Pre	++	++	++		2	40 ml A+
117-H	10	+	+	+	X	42	1st inf 12-18-53
2-3-54	60	0	0	0		18	Died 2-4-54
FV-4	Pre	++	++	++		5	35 ml 0+
113-H	10	0	0	0	X	∞	1st inf 12-23-53
2-5-54	60	0	±	+		6	Well 2-6-54
FV-5	Pre	+	+	++		3	35 ml. 0+
123-H	10	+	+	++	X	∞	1st inf 1-21-54
2-15-54	60	dead				X	Died 1 hr. post-inf
FV-6	Pre	0	0	+		7	30 ml A+
124-H	10	0	0	0	X	∞	1st inf 2-10-54
3-2-54	60	0	0	±		∞	Died 3-3-54

these eight animals, six died within 6 hours, one was well and free of disease, while one animal had a period of acute renal disease lasting seven days during which the blood urea nitrogen rose to 65.5 mg. per cent and then returned to the pre-infusion value of 19.4 mg. per cent. Of the entire group of fourteen animals receiving human blood for the second time, eight died, three experienced a period of acute renal disease, and three recovered with no evidence of renal failure.

[illegible]

direction. Analyses of the urine of animals receiving human blood for either the first or the second time failed to show the presence of proteolytic enzymes or an antiproteolytic factor.

DISCUSSION

The data presented here indicate that the infusion of human blood to sensitized dogs is associated with the production of acute renal disease or death in most of the animals. The phenomenon of anaphylaxis or any antigen-antibody reaction has been felt to be associated with the activation of circulating proteolytic enzymes. With the administration of human blood to sensitized dogs, there is found some activation of the plasma proteolytic enzymes when measured against fibrinogen or casein. The differences in proteolysis between the first and second infusions are not as great as had been expected and we are unable at present to demonstrate a relationship between the activation and the clinical symptoms or subsequent renal disease. The failure of whole blood clotting in this group of animals is not well correlated with the ability of fibrinolysin to digest fibrinogen or casein. It is interesting to note the lack of correlation between increases of plasma proteolytic activity for casein and for fibrinogen in these tests. Investigation of this relationship has not yet been completed.

CONCLUSIONS

1. Initial infusions of human blood in non-sensitized dogs produce mild irregular variations in plasma proteolytic activity.

2. Infusions of human blood into sensitized animals produce transient increases in plasma proteolytic activity toward casein and less consistent increases of such activity toward bovine fibrinogen.

3. The increased plasma proteolytic activity observed in sensitized animals cannot be correl [illegible] evelopment of renal impairment,

4. No variations [illegible] tivity or of plasma antiproteolytic activity are observed in this group of animals in response to the infusion of human blood.

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The data are recorded as optical density readings in the colorimeter. These have been multiplied by 100 to give arbitrary units of caseinolytic activity, since in the range used the relation of proteolytic activity to optical density increase approximates a straight line

Plasma Antiproteolytic Titers. Varying dilutions of whole plasma are incubated with a solution of trypsin. The amount of gelatin digested by the mixture is then compared with an uninhibited digestion curve. The inhibition of tryptic digestion by plasma can then be measured. These data are not reported in detail since there was no change in the antitryptic activity in any animal.

Urine Proteolytic Activity. Gelatin and fibrinogen digestion were measured by tests similar to those used for plasma. These data are not reported in detail since we did not find any urinary proteolytic activity.

Urine Antiproteolytic Activity. Urine antitryptic activity was measured by a test similar to that used for plasma. Trypsin inhibition was not observed in any urine sample.

RESULTS

The intravenous infusion of 100 ml of human blood to a 9 to 11 kg. dog is accompanied by a brief period of hypotension, minimal tachycardia, slight pallor and, rarely, vomiting This period of reaction lasts about 15 minutes and upon termination of the infusion the animal appears to be well. Eight dogs receiving their first infusion of human blood have been examined for proteolytic activity in the plasma. Data are presented in Table 1 to show the extent of this activation. It is apparent that there is little, if any, activation of fibrinolysin when fibrinogen is used as a test substrate. Using casein as the substrate, slight activity is found The greatest increase in casein activity is 12 units. In one of these animals, 134-H, the 10 minute sample of whole blood did not clot for 75 minutes

The infusion of 30 to 45 ml. of human blood to these same animals three weeks after the first infusion of human blood produced severe collapse, tachycardia, vomiting, retching, tenesmus, bloody diarrhea, and anuria for several hours. Fourteen dogs receiving the second infusion of human blood have been examined for proteolytic activity in the plasma. Data are presented in Table 2 to show the extent of this activation. It is apparent that there is some proteolytic activity when tested against fibrinogen in the 10 or 60 minute sample in five of the fourteen animals. In three of these the activation is adequate to prevent clot formation in all tubes of the fibrinogen test. In five of the animals of this group casein was used as a test substrate and the infusion of human blood was followed by an increase in casein units ranging from 7 to 17 units.

Chi square analysis of fibrinolysin activation when tested against fibrinogen shows that there is no significant difference between groups I and II With respect to activity against casein, group I shows a nonsignificant increase in the post-infusion activity, while in group II this is significant.

In the group II animals, all the pre-infusion casein activities are high We are unprepared to say whether this reflects true initial activity in a sensitized animal or whether it is a contaminant present in the casein substrate of that particular casein preparation.

Whole blood clotting time was longer than 2 hours in at least one of the post-infusion blood samples in eight of these fourteeen animals Of

and poly-unsaturated fatty acid composition by the American Oil Chemists' Society methods Cd 1-25 and Cd 7-48, respectively. Melting points were determined by the method of Hawk, Oser and Summerson.[10]

The two groups were placed in separate pens equipped with self-feeders. *One group was fed a diet rich in soybean oil and the other group a ration* rich in hardened coconut oil. The detailed composition of the rations is listed in Table 1. After four months on the diets, specimens of the subcutaneous back fat were obtained from each hog and analyzed as before. The hogs were then subjected to general hypothermia, attempting to simulate as closely as possible conditions existing in the clinical use of cold in cardiovascular surgery.

Table 1. Composition of Diets

	BREWER'S RICE	TANKAGE (60% PROTEIN)	ALFALFA MEAL	SALT	HARDENED COCONUT OIL	SOYBEAN OIL
Coconut group	66 5%	15%	8%	0.5%	10%	.
Soybean group	66 5%	15%	8%	0.5%	..	10%

Note: Each ration included supplements of mineral mixture and Aureomycin.

In preparation for the cooling process each animal was anesthetized with sodium pentobarbital in doses of 20 mg. per kilogram by intraperitoneal injection. An endotracheal tube was then inserted and the animal was immersed in an ice bath. A deep tissue temperature attachment for a thermocouple was inserted into the paraspinal fat for the purpose of recording variations in fat temperature, and a constant recording rectal thermometer was inserted. Additional pentobarbital was given intravenously in small quantities as needed to control restlessness and shivering. The rectal temperature was lowered to 28°C., following which the animal was removed from the ice bath and exposed to room air for one hour. Warming was accomplished by immersion in a water bath maintained at 45°C. Two weeks following the hypothermia procedure multiple biopsies of the subcutaneous adipose tissue were obtained and the specimens prepared for histologic examination.

RESULTS

Clinical examination of all animals after removal from the ice revealed no detectable gross abnormality in the subcutaneous tissue of the hogs of the soybean group, whereas definite areas of nodularity were palpable in some members of the coconut series. In the period subsequent to hypothermia the hogs of the soybean group remained active and maintained a good appetite; conversely, the members of the coconut series were lethargic and demonstrated profound anorexia for a period of three to four weeks.

The complete results of the chemical analyses of the adipose tissue specimens obtained before and after four months' ingestion of the special rations are listed in Table 2. In the coconut group the total unsaturated fatty acids and the iodine numbers showed a striking diminution from the control values, accompanied by a commensurate elevation in the total saturated acids and melting point. A relatively small change occurred in the composition of the subcutaneous fat of the animals fed soybean oil (Fig. 1).

Histologic examination of the specimens obtained from the coconut oil

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EXPERIMENTAL PRODUCTION OF SUBCUTANEOUS FAT NECROSIS BY GENERAL HYPOTHERMIA*

Relation to the Chemical Composition of Fat

JESSE E. ADAMS, JOHN H. FOSTER, WALLACE H. FAULK, AND H. WILLIAM SCOTT, JR.**

Induced general hypothermia, largely due to the stimulus provided by the fundamental research of Bigelow,[1,2] has become increasingly important as an adjuvant in cardiovascular surgery Subcutaneous fat necrosis is a postoperative complication of the clinical use of general hypothermia, as currently employed in cardiovascular surgery, which can be directly attributed to the induction of the cold state[4] Thus far, in our experience, this phenomenon has occurred only in infants. It has been hypothesized that a lower percentage of unsaturated fatty acids associated with a higher solidification point in the subcutaneous fat of infants may explain the increased susceptibility of this age group to fat necrosis following hypothermia.[14] To test this hypothesis an experiment was planned to determine the effect of cold on the adipose tissue of two groups of animals whose fat composition had previously been altered by diet in such a way as to produce a relatively low percentage of unsaturated fatty acids in one group and a relatively high percentage in the other

EXPERIMENTAL PROCEDURE

Twelve pure-bred Duroc pigs of weanling age were selected from two litters which had a common sire and which had been farrowed within 48 hours of each other. The animals were divided into two groups of six each, and in the group formation they were divided in such a way as to give the best balance possible with respect to litter origin. When the animals had attained the age of eight weeks, approximately 5 grams of subcutaneous fatty tissue were obtained from each by biopsy from the right jowl, and these specimens were rapidly frozen with dry ice to prevent oxidation of the fat. A portion of each specimen was prepared for microscopic examination and the remainder was subjected to chemical analysis After desiccation and extraction of the specimen, the fat samples were analyzed for iodine value

* ... of Medicine, Nash- ... h grant A-593 from ... ational Institutes of

** ... O'Connor of the Southern Regional Research Laboratory for the chemical analyses of fats, and to the Proctor & Gamble Co. for supplying the oils used.

group two weeks subsequent to hypothermia demonstrated definite fat necrosis to be present in each animal (Fig. 2). Careful examination failed to reveal evidence of fat necrosis in any of the specimens obtained from the soybean oil group subsequent to hypothermia. All sections were reviewed by Dr. Ernest Goodpasture, Head of the Department of Pathology at Vanderbilt University School of Medicine.

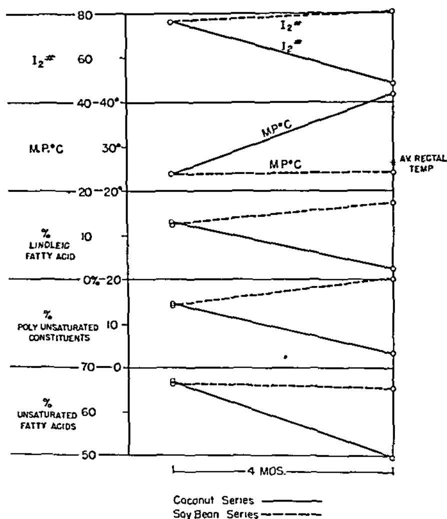


Fig. 1. Chemical determinations before and after special rations.

DISCUSSION

Subcutaneous fat necrosis has been observed and verified histologically in this hospital postoperatively in two infants whose rectal temperatures were lowered to the range of 28° to 31°C. as an adjuvant to cardiac surgery. Its occurrence was strongly suggested in a third infant who developed tender lumps under the skin after similar exposure to hypothermia, but histologic proof was not obtained in this case. We have recently learned of another instance of fat necrosis which occurred in another clinic after the use of hypothermia for cardiac surgery in an eighteen month old infant.[9]

Subcutaneous fat necrosis is an infrequent complication of hypothermia as currently used for cardiac surgery, but there is considerable evidence in

Table 2 Average Determinations of Fat Analysis of 12 Animals before and after Special Diets

DIET	IODINE NUMBER		MELTING POINT		TOTAL SAT. FA		TOTAL POLY-UNSATURATED NON-CONJUGATED CONSTITUENTS		LINOLEIC ACID		FAT NECROSIS
	INITIAL	FINAL	INITIAL	FINAL	INITIAL	FINAL	INITIAL	FINAL	INITIAL	FINAL	
Soybean (6 animals)	76 3	80 3	24°C.	24 1°C	27 68%	30 20%	14 39%	20 04%	12 83%	17 19%	0%
nut animals)	76 9	48 3	24°C	41 4°C	27 54%	46 05%	14 81%	3 31%	13 18%	2 77%	100%

low percentage of unsaturated fatty acids in infant fat may be an important etiologic factor in the occurrence of subcutaneous fat necrosis in this age group following exposure to cold.

We used swine in this project because of the ease with which their fat can be changed by variation of diet and because their size permits multiple biopsies to be obtained. A fundamental difference exists, however, between the subcutaneous fat of humans and pigs; human subcutaneous fat tends to become more unsaturated with increasing age while swine subcutaneous fat tends to become more saturated. Ellis[5, 8] found that the subcutaneous concentration of linoleic acid, a poly-unsaturated, non-conjugated constituent of fat, followed the dietary intake very closely and he regarded this acid as the most important of the food fatty acids from the point of view of affecting the degree of unsaturation of stored fat. He and his co-workers maintained groups of hogs on different diets, some composed of a high per-

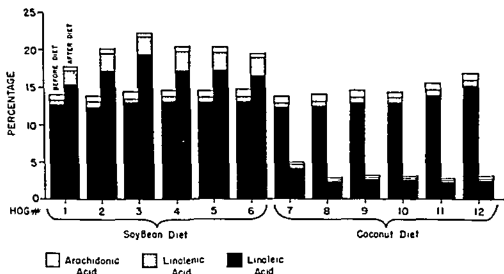


Fig. 3. Poly-unsaturated non-conjugated constituents of subcutaneous fat of hogs.

centage of saturated fatty acids, and others on diets rich in unsaturated fatty acids. He found that on a diet of soybeans, the linoleic fatty acid was 30 6 per cent of the stored fat, while on a hardening diet rich in saturated fatty acids, the linoleic fatty acid concentration was only 1.9 per cent of the stored fat. The oil obtained from soybeans has an iodine number of 124 to 133 and contains approximately 52.6 per cent linoleic acid. This product seemed ideal to produce a depot fat containing a high percentage of unsaturated fatty acids. Refined, hardened coconut oil, with an iodine number of 1, and containing only 2 5 per cent linoleic acid, was a readily available product which it was felt would produce a depot fat containing a relatively small amount of unsaturated fatty acids.

It will be noted in Table 2 that no significant change from control values occurred in the fat of the hogs fed the soybean ration for four months despite the fact that soybean oil contains a high percentage of unsaturated acids. We feel that this is explained by the tendency of hog fat to increase in degree of saturation as the hog matures and by the fact that brewer's rice tends to produce saturated fat. The change in the average melting

the literature to suggest that necrosis of depot fat may be caused by exposure to cold. Hocksinger[12] and Haxthausen[11] have reported cases of subcutaneous fat necrosis in children following exposure to low temperatures. Lemez[13] produced sharply demarcated areas of fatty tissue necrosis in newborn and young infants by application of ice and by brief freezing with ethyl chloride.

Haxthausen[11] felt that the tendency of infants to develop fat necrosis

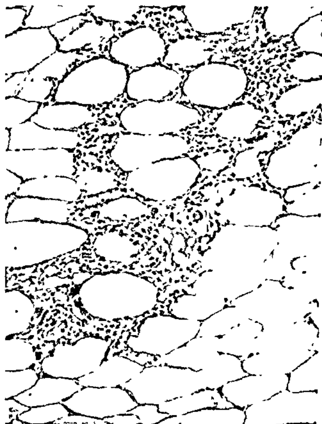


Fig 2 Photomicrograph of subcutaneous fat of hog on coconut oil diet, showing fat necrosis

depended upon the particular chemical composition of their fat, i e., a relative reduction of the amount of unsaturated fatty acid in the fat cells.

His theory is partially confirmed by Channon and Harrison,[3] who reviewed the literature concerning analyses of subcutaneous fat and found that the melting point of subcutaneous fat of infants was much higher than that of adults, accompanied by a lower iodine number of the subcutaneous fat of the infant fat The iodine number is an index of the degree of unsaturation of fat and therefore varies inversely with the melting point and the percentage of saturated fatty acids Thus, it would appear that infant fat should contain a higher percentage of saturated fatty acids (and conversely a lower percentage of unsaturated fatty acids) than that of adults. Since the recent introduction of the spectrophotometer, there have been to our knowledge no published comparative analyses of the composition of infant and adult fat. It seems logical, however, to assume that the presumed

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point in the group is in keeping with the negligible change in unsaturated acids, amounting to only 0.1°C. The distinct decrease in total unsaturated acids from control values in the coconut oil group is largely a reflection of the markedly decreased values for linoleic acid (Fig. 3). As would be expected, the changes in unsaturated acid values are reflected in the elevated melting point values and decreased iodine numbers The subcutaneous temperature determinations, on the average, were 4° to 5°C. below the rectal temperatures. During the hypothermia procedure the average of the lowest rectal temperatures to which the soybean animals were cooled just exceeded the average melting points of their fat, whereas the average rectal temperature of the coconut group was below the average melting point of their fat (Fig. 1). During hypothermia, the temperature of the adipose tissue of the animals in the coconut oil group apparently fell below the solidification point and as a result, fat necrosis developed, whereas the subcutaneous tissue of the soybean oil series, although subjected to the same temperature, did not solidify and hence was not damaged. It appears logical to assume that the fat of the soybean hogs was protected from injury by virtue of containing more unsaturated fatty acids with a consequent lower melting point Since the relative amounts of linoleic acid constituted the principal difference between the two series, it strongly suggests that this highly unsaturated acid played the major role in protecting the soybean fat from damage.

In view of our findings and observations of investigators such as Lemez and Haxthausen, it seems reasonable to speculate that young children develop fat necrosis as a result of cold because their fat is highly saturated. The intriguing question then arises as to whether infants might be protected from fat necrosis by administration of a highly unsaturated lipid, for example, linoleic acid.

It should be emphasized that we do not feel that fat necrosis occurring secondary to controlled hypothermia as now utilized represents a contraindication to use of this promising adjuvant to cardiovascular surgery

CONCLUSIONS

1 Subcutaneous fat necrosis may occur as a complication of induced general hypothermia and this phenomenon is peculiar to young children.

2. Subcutaneous fat necrosis can be produced in swine by the general application of cold

3 Swine which have ingested large quantities of unsaturated fats are protected from subcutaneous fat necrosis secondary to lowered body temperature because their fat is rich in unsaturated fatty acids and, as a result, has a low melting point.

4. Subcutaneous fat necrosis following induced general hypothermia in young children probably occurs because their fat contains a relatively low percentage of unsaturated fatty acids, and as a consequence, has a relatively high melting point.

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STEROIDS AND CANCER

INTRODUCTION

FRANCIS D. MOORE

Among the many fields of surgery which are advancing rapidly at the present time, that of "steroids and cancer" comes close to leading the list. Increased knowledge of the steroid hormones and of surgical endocrinology has brought us progressively closer to an understanding of the bodily reactions to stress in general and to surgery in particular. In addition, certain endocrine glands have been found to be of importance in the regulation of the growth of tumors; in the present state of our knowledge, the hormones most intimately involved are likewise steroids. Increased knowledge of these [illegible] laily treat- [illegible] with men- [illegible] Forum in Atlantic City in 1954.

The growth of any scientific study depends upon increased accuracy of observation. In endocrinology and biochemistry, this usually means quantitative methods of measurement. In the steroid field, most of the recent advances are based on increased accuracy in methods of measurement. The studies of Hume and Nelson, of Cooper, Roberts, and Touchstone, of Jabbour and Hardy, of Steenburg, and of Watne are based on quantitative measurements of adrenal function under various circumstances. Nelson and Samuels have described a practical method for the measurement of steroid hormones in the blood, and Hume and Nelson have developed an accurate assay for ACTH in the blood. The studies on adrenocortical function in surgical shock indicate that the adrenal in shock puts out its hormones at tremendously increased concentration; the blood flow itself is reduced somewhat. The result is an elevated output of steroid in the shocked organism, despite the decrease in blood flow. The studies of Cooper, Roberts, and Touchstone are based on paper chromatographic separation of steroids, and they indicate that compound F is the most abundant steroid produced by normal human adrenals. Steenburg's studies are likewise based on the blood steroid level in surgical patients, and demonstrate a very sharp rise in blood steroid at the outset of operation, or even with anesthesia alone. The method measures the free steroids and these drop off sharply within a few hours, presumably as a result of conjugation by the liver.

Studies of urinary excretion of steroids, as exemplified by those of Jabbour and Hardy, and of Watne, continue to demonstrate extremely important changes. It is likely that the urinary excretion of steroid hormones over a period of days following injury may be a more accurate index of the total magnitude of the increased adrenocortical function, the blood curve remaining a more precise index of the timing and physiologic mechanism involved.

Turning to steroidal effects on cancer, Patterson demonstrates again that the cheek pouch of the hamster makes a good spot for the growth of human tumors. The growth of human tumors in the experimental animal may ultimately provide the best way of measuring the patient's potential benefit

iodine is entirely collected to the area of the graft. There are very few recorded instances in the world literature of true survival and function of homotransplanted tissue. This must be recorded as one, and it involves not one tissue but two. One cannot help but think of Dr. Halsted's old rule that for transplanted tissues to take, there must be a need! In addition, the potential use of embryonal tissue is again emphasized.

from various therapeutic agencies. It is of interest to note that Patterson's transplants require the administration of cortisone, for their take and growth. This probably has something to do with the abolition of normal immune responses by the glucocorticoids.

Allen, Vasicka, and Sturgis have shown that when mouse mammary carcinoma is grown in vitro the nucleic acid synthesis in the tumor may be affected significantly by estrogens and testosterone. This is another way of measuring quantitatively steroic [illegible]

The work of Shapiro takes a [illegible] transplantable mouse mammary [illegible] are non-steroidal in character, but which probably derive their biological activity from configurational similarity to substrates naturally occurring in the patient.

Immune aspects of cancer have fascinated investigators for many years. Other than certain specialized observations in rodent tumors, there is comparatively little information available. The studies of the Grahams indicate that there are circulating antibodies to certain tumor antigens, as judged by complement fixation. Schrek and Preston have demonstrated acquired immunity to tumor inoculation in a new setting, namely the Sprague-Dawley rat reacting to a reticulum-cell lymphosarcoma. Evidently the immune property was circulating and could be demonstrated in the serum.

The importance of the lymphatics in the transport of tumors treated surgically is indicated by the continuing interest in lymphatic function. Ju, Blakemore, and Stevenson have developed a lymphatic function test, depending upon the transport of iodinated albumin. Thomas has studied lymphatic dissemination through the use of radioactive gold, measuring tissues directly, and also scanning the patient externally. Rasmussen-Taxdal, Ward, and Figge have studied lymphatics and cancer tissue by their fluorescence under the administration of hematoporphyrins. Strug, Leon, and Cohn have also been interested in lymphatic function and are studying the staining of lymphatics by direct sky blue. Many dyes are selectively transported in lymphatics, but this one appears to be particularly valuable and may even be useful intraoperatively.

The work of Galante, Rukes, Flanagan, Forsham, and Wood is of exceptional interest. They have shown that when adrenal venous blood is shunted into the portal circuit, the effects of adrenalectomy are produced, suggesting that a normal liver removes adrenal steroids from the blood so rapidly that infusion into the portal circuit is tantamount to removal from the body.

And finally, mention should be made of one of the most spectacular papers of the entire Clinical Congress, although it may not have too much to do either with steroids or with cancer! This was the work of Sterling and Goldsmith of Philadelphia. They presented a very well documented and well studied case in which the transplantation of a fetal thyroid with attached parathyroids to a myxedematous hypoparathyroidal patient resulted in complete endocrine rehabilitation To clinch their case, they brought the patient herself to the Forum meeting and I believe that all of us were much impressed.

The transplant was taken out en bloc from a 21 day old infant. Direct vascular anastomoses were carried out on the groin. Evidently the entire transplant has functioned. The patient requires no more thyroid and no more treatment for hypoparathyroidism. In addition, administered radio-

For this reason a technique has been developed for the direct measurement of corticoids in the adrenal venous blood of the intact dog.[5,6] A study has been made of the rate of blood flow through the adrenal, the minute output of corticoids, and the concentration of corticoids per milliliter of adrenal venous blood under the following conditions: (1) during operative trauma; (2) in oligemic shock, created by bleeding the traumatized, anesthetized animal; (3) in the convalescent period following surgical trauma.

METHODS

Adult male mongrel dogs varying in weight from 14 to 26 kilograms were used for all experiments. A total of 55 experiments was carried out in 40 animals. The animals were divided into 5 groups:

1. Normal dogs subjected to acute surgical trauma under Nembutal or ether anesthesia (24 dogs).

2. Normal dogs from group 1 whose adrenal venous blood corticoid output was followed in the convalescent period after the acute trauma, as well as on the day of the trauma (6 dogs).

3. Normal dogs subjected to operative trauma (adrenal cannulation) and controlled hemorrhagic shock (6 dogs which were also in group 1).

4. Hypophysectomized dogs subjected to operation and hemorrhagic shock (16 experiments on 13 dogs). This group was further subdivided into the following categories:

a. Chronic hypophysectomized dogs not receiving ACTH.

b. Chronic hypophysectomized dogs which, following hypophysectomy, received bi-daily injections containing 20 units of a long-acting ACTH preparation until 18 hours before the hemorrhage. No ACTH given during the operative trauma and shock periods.

c. Same as (b), but receiving a constant intravenous infusion of ACTH during the operation and hemorrhage.

[illegible] dogs receiving a constant intravenous in-[illegible]na and shock periods.

[illegible]lly sympathectomized dogs subjected to operation and hemorrhage (3 dogs).

Cannulation of the adrenal vein was carried out by a technique to be described elsewhere.[6] In brief, this consisted of cannulating the lateral portion of the right lumbo-adrenal vein with a polyvinyl catheter which was brought to the outside through a stab wound (see Figure 1). A "choker" made of polyethylene was placed around the adrenal vein between the adrenal and the entrance of the vein into the vena cava. It was brought out through the operative wound, so that it could be manipulated from the outside. With the choker relaxed, the adrenal secretions were released into the vena cava in the normal fashion. With the choker pulled up, the adrenal blood flowed to the outside through the cannula, and was collected in a graduated tube over a measured time interval (usually one minute). The quantity of blood flowing out during this period indicated the adrenal blood flow. The 17-hydroxycorticosteroids contained in the adrenal venous blood samples were measured by the method of Nelson and Samuels.[9] In the dog these consist principally of hydrocortisone.[10] The cannula was filled with heparinized saline, containing 4 mg. of heparin per 10 cc., and sealed with a special stainless steel plug between samples. The animal was not heparinized.

ADRENAL CORTICAL FUNCTION IN SURGICAL SHOCK*

D. M. HUME AND D. H. NELSON**

Very few direct experimental data have been published regarding adrenal cortical function in shock. Vogt[12] collected adrenal venous blood from a series of anesthetized, heparinized dogs
the blood by bioassay. It was noted tha
sure or in blood flow through the gland
output of cortical hormones. The author pointed out, however, that the method used for determination of corticoids was of limited accuracy, and could measure only threefold increases or reduction to one-third of the basal value

Hayes[4] states that reduced renal plasma flow in shock suggests that the adrenal blood flow is also reduced, leading to a "subsequent possible reduction in the amounts of adrenocortical steroids available in the circulation per unit time" He concludes that. "In some human cases because of hemodynamic failure in the shock syndrome, a temporary period of relative hypoadrenocorticism may supervene This complication may be an important contributor to the perpetuation of the shock state with an irreversible outcome."

If rats are subjected to graded hemorrhage there is a depletion of adrenal ascorbic acid, suggesting that corticoid secretion from the adrenal may have occurred.[7] If severe bleeding is carried out abruptly, however, rats[11] or dogs[1] may die in shock with little or no reduction in carbonyl-containing lipoid of the adrenal cortex, suggesting that adrenal corticoid secretion may not have occurred under these circumstances. It is postulated that this may be a consequence of the abrupt reduction in adrenal blood flow.[1] Preliminary bioassays of adrenal venous blood in dogs so bled were said to show "no increase in corticoid secretion during hemorrhagic shock."[13] It is not stated, however, to what trauma the animal was subjected in order to obtain the sample of adrenal venous blood. It may have been that "maximal" adrenal corticoid secretion had already been initiated by this trauma.

Franksson and Gemzell[2,3] have studied peripheral blood 17-hydroxycorticosteroid levels in 5 patients who developed shock during the operative or postoperative period. In all cases very high blood corticosteroid levels were obtained, even prior to death, when shock had lasted for several days In two patients who developed shock immediately following traumatic injury, the blood corticosteroid levels seemed to be low during the immediate shock period, rising the next day when the patients were out of shock. They felt that the explanation for this might be on the basis of circulatory disturbances of the pituitary, leading to a decreased secretion of ACTH.

Peripheral blood and urinary steroid levels are not always adequate to define adrenal cortical activity in injury, because of changes in conjugation and excretion of corticosteroids that accompany trauma and anesthesia.

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A plastic blood collecting bag* was connected to one arterial cannula, and shock was induced by allowing the animal to bleed into this bag. The amount of blood lost was measured by weighing the bag. Bleeding was usually allowed to continue until the mean arterial pressure had reached a level of 25 to 45 mm. Hg. A determination of adrenal blood flow and adrenal venous blood corticoid output was made at various pressures between this level and normotensive levels, both during the bleeding and during the replacement of the blood. The blood was replaced intra-arterially by applying pressure to the plastic blood bag or, in some instances, by injection through a syringe connected to the bag through the side arm of a three-way stopcock. The animals were allowed to remain in shock for periods varying from 10 minutes to 79 minutes.

RESULTS

Group 1: Normal Dogs Subjected to Acute Surgical Trauma under Nembutal or Ether Anesthesia; Corticoid Output during and up to 4 Hours after Trauma. a. In 11 dogs operated under ether anesthesia the average adrenal blood flow from one adrenal varied between 4.5 and 7.2 cc. per minute, with an over-all average for all dogs of 6.0 cc. per minute. The adrenal venous blood 17-hydroxycorticosteroid output for one adrenal varied between 9.0 and 22.2 gamma per minute, with an over-all average for all dogs of 12.7 gamma per minute.

b. In 13 dogs operated under Nembutal anesthesia, the average adrenal blood flow varied between 1.3 and 7.0 cc. per minute, with an average for all dogs of 3.6 cc. per minute. The adrenal venous blood 17-hydroxycorticosteroid output varied between 2.4 and 20.8 gamma per minute, with an average for all dogs of 9.4 gamma per minute.

Group 2: Normal Dogs from Group 1 Whose Adrenal Venous Blood Corticoid Output Was Followed in the Convalescent Period. In 6 dogs samples of adrenal venous blood were obtained on the day of operation and 1 to 5 days thereafter. By the morning after the operative trauma, the corticosteroid output had fallen to low levels, although the adrenal blood flow remained about as it had been during the operation. After the second post-trauma day the corticosteroid output averaged from 0.0 to 2.0 gamma per minute. An occasional isolated value as high as 20.0 gamma per minute was seen. An infusion of ACTH in the unanesthetized resting dog produced values as high as 23.0 gamma per minute, which is comparable to the highest values seen with operative trauma. The secretion of ACTH from the pituitary in the convalescent animal is apparently sporadic, in contrast to the continuous high levels seen with operative trauma.

Group 3: Normal Dogs Subjected to Operative Trauma and Hemorrhagic Shock. The results in this group are summarized in Table 1. It may be seen that in dog No. 3, where the bleeding was only moderately rapid, there was no decrease in corticoid minute output even when the blood pressure fell to 40 mm. Hg and the adrenal blood flow fell to 0.5 cc. per minute. The high level of corticoid secretion was maintained by a sevenfold increase in concentration of corticoids per cubic centimeter. Dog No. 36 was given a constant infusion of ACTH and was bled slowly over a long period of time. No decrease in corticoid output was noted. Dog No. 48 was bled rapidly, transfused after a period in shock, and bled rapidly again. No decrease in corticoid secretion was noted in either instance. At the conclusion of the

* Fenwal Laboratories.

The operative procedure for adrenal vein cannulation was carried out under aseptic conditions in those dogs used for chronic studies during the convalescent period, and in the chronically acute experiments were done without sterile were performed via the transbuccal route.[6, 8] sympathectomy consisted in dividing, below the diaphragm, the greater, lesser, and least splanchnic nerves, together with the last thoracic and first 3 lumbar sympathetic ganglia and connecting chains.

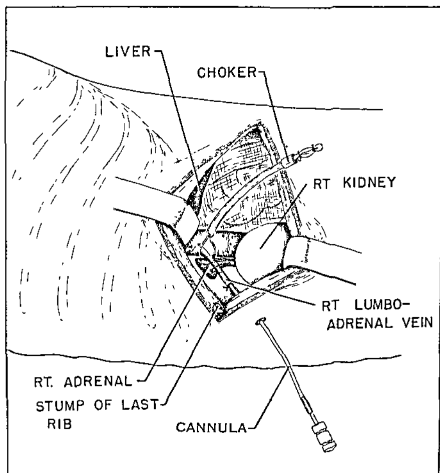


FIG. 1. The method for obtaining adrenal venous blood samples is illustrated Adrenal blood enters the lumbo-adrenal vein to flow into the vena cava when the choker is relaxed, and out the cannula when the vein is constricted off by pulling up on the choker The incision is made through the bed of the last rib, after resecting it

In the shocked animals, plastic cannulae were placed in both femoral arteries, one for recording the blood pressure, and the other for bleeding the animal. When a constant ACTH infusion was given it was administered through a cannula in a femoral vein.

A strain gauge* was connected to one arterial cannula, and continuous measurement of the mean blood pressure was obtained by feeding the output of the strain gauge circuit into a recording potentiometer.†

* Statham Instrument Co.

† General Electric photoelectric potentiometer recorder type CE-5.

experiment an additional 250 cc. of blood was removed, and it was only at this point—after the loss of 59 cc. of blood per kilogram—that the adrenal blood flow and corticoid output fell to very low levels.

Dog No. 110 was subjected to severe anoxia prior to the hemorrhage by the production of a pneumothorax. A marked decrease in 17-hydroxycorticosteroid secretion occurred during the period of shock induced by hemorrhage. When the lost blood was given back there was a rise in corticoid secretion almost to pre-bleeding levels. The blood pressure never rose above 60, however, and the animal died 5 minutes after the transfusion.

Dogs Nos. 108 and 111 were bled rapidly. Both of them had rather low initial corticoid levels. Both dogs showed great increases in minute corticoid secretion in shock. The blood pressure in dog No. 108 did not fall below 64, but the corticoid output at this level averaged 21.1 gamma per minute, compared to a pre-bleeding average of 9.0. The values remained high during and immediately after transfusion, but 16 minutes after all the blood had been replaced the corticoid secretion was down below the pre-bleeding levels.

Dog No. 111 showed a decrease in corticoid output during the 5 minutes of the actual blood withdrawal, from a pre-bleeding average of 5.0 gamma per minute to a low of 2.9 gamma per minute at a blood pressure of 35 mm. Hg, and an adrenal blood flow of 0.2 cc. per minute. No blood was given to the animal at this point, but the blood pressure rose over the next two minutes to 58, the adrenal blood flow increased to 2 0 cc. per minute, and the corticoid minute output jumped to 23.8 gamma per minute. During and immediately after the period of blood replacement, values of over 30 gamma per minute were noted. Ten minutes after all the blood was replaced the corticoid output had decreased to levels around 10 gamma per minute.

[illegible] ed to Operation and Hemor- [illegible] Dogs Not Receiving ACTH. [illegible] response to operative trauma, acute hemorrhage, or administration of a single dose of ACTH intravenously.

b *Chronic Hypophysectomized Dogs Maintained on ACTH after Hypophysectomy, but Receiving No ACTH during the Operative and Shock Period* No significant corticoid secretion was noted in response to operative trauma or acute hemorrhage. Intravenous administration of ACTH was followed immediately by corticoid secretion as great as that seen in the normal do [illegible] iggest that hemorrhagic sh [illegible] n the absence of ACTH.

c *Same as (b), but Receiving a Constant Intravenous Infusion of ACTH during the Operation and Hemorrhage.* Eight experiments were performed in this category. Both ether and Nembutal anesthesia were used. Following cannulation of the adrenal vein an ACTH infusion was begun, and several samples of adrenal venous blood were obtained. The animals were then bled, maintained in shock for a time, and transfused. In none of the animals was there a decrease in corticoid output in shock, except for an isolated sample obtained at a time when the shock was so severe that the adrenal blood flow had all but ceased. In five of the 8 experiments there was a slight to very marked increase in minute corticoid output during shock and in the immediate post-transfusion period. This could not be related to changes in ACTH secretion because the blood ACTH was being maintained at a con-

Table 1. Adrenal Venous Blood Flow and 17-Hydroxycorticosteroid Output from One Adrenal Gland of Normal Dogs before and after Hemorrhage

			ADRENAL BLOOD FLOW (CC /MIN) AND 17-HYDROXYCORTICOSTEROID OUTPUT (GAMMA/MIN.) AT VARIOUS BLOOD PRESSURE LEVELS											
			PRE-BLEEDING		70-90		60-70		50-60		40-50		25-40	
DOG	NO CC BLED	WT IN KG	FLOW	17-OH	FLOW	17-OH	FLOW	17-OH	FLOW	17-OH	FLOW	17-OH	FLOW	17-OH
3	360	11 2	3 5	11 0	1 5	18 0							0 5	16 0
48*	580 750	17 0	6 2	9 5			1 8	10 4	2 3	12 6	1 6	10 0	0 4	0 1†
108	350	14 5	3 1	9 0	1 0D 4 4U	9 2D 21 1U	1 9	21 1						
110	225	14 0	2 6	21 6			1 5D	3 9D	2 8U	18 6U	1 6	5 7	0 5	0 8
111	120	13 0	2 6	5 0	2 4U	24 8U			2 0U	23 8U	0 4D 0 5U	2 9D 7 3U	0 2	2 9
36	725	16 5	11	13 3	Bled slowly over 2 hours and 10 minutes There was no change in corticoid output, the average for the values obtained after hemorrhage being 14 2 gamma per minute									

B P (MM HG) ←

*Ether anesthesia All other dogs were under Nembutal anesthesia Bled twice All values averaged together

†This single value after 1000 cc of blood removed

Prior to hemorrhage the animals were subjected to an operative trauma (insertion of the adrenal cannula) under anesthesia Dog No 110 was rendered anoxic in addition, by the creation of a pneumothorax In dog No 3, the bleeding was accomplished in two equal stages separated by a 10 minute period. In dogs Nos 48, 108, 110, and 111, the bleeding was carried out rapidly over 3 to 7 minutes

The pre-bleeding values are the averages of the values obtained after the operative trauma, but before the hemorrhage Where there were several values for a given dog at any one blood pressure level they were averaged together Sometimes a value, or series of values, was obtained at an intermediate blood pressure level both while the blood pressure was falling due to the hemorrhage, and when it had begun to rise again as a consequence of compensatory mechanisms or transfusion The values obtained when the blood pressure was falling are marked with a "D," and those obtained when it was rising are marked with a "U"

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THE INFLUENCE OF ALTERATIONS IN ADRENAL CORTICAL FUNCTION ON THE TOLERANCE TO TRAUMA*

ANDREW KIRSTEINS

During the last twenty years voluminous literature has accumulated in the field of corticosteroids and their effects on shock and allied conditions. However, the claims for benefit are about equal to the claims that effects are negligible or harmful. Therefore, to evaluate the role of increased or decreased adrenal cortical hormone levels in tolerance to trauma and hemorrhagic shock, an experimental study using 50 dogs and 180 rats was carried out.

The first part of this report deals with steroid therapy of dogs subjected to splenectomy and portal vein occlusion; the second part contains data on rats subjected to free bleeding after unilateral transection of the femoral artery and vein.

To induce a reproducible shock in dogs a method was sought which (1) would consistently produce shock of a similar degree in individual animals, (2) was not dependent upon human error and (3) would uniformly lead to death. Acute portal occlusion with consequent pooling of blood in the splanchnic bed was found to be a consistent and reliable method for production of shock. The amount of blood lost in the splanchnic bed represents 5 to 6 per cent of the animal's body weight, an amount sufficient to account for death.[1] An additional advantage of this method was thought to lie in the fact that the rate of blood loss into the bowel wall would depend to some degree upon vascular tonus and capillary permeability, two factors which may be influenced by the adrenal cortex.[2] To reduce further the mechanical sequestration of blood into the splenic sinusoids a splenectomy was performed preceding each portal ligation. The survival of splenectomized dogs

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stant (and very high) level. Supplementary injections of large amounts of ACTH intravenously did not produce similar increases. It would appear, therefore, from the studies on normal dogs, that severe hemorrhage can elicit a marked release of pituitary ACTH. The studies on the hypophysectomized dogs suggest that in shock there may be an increased responsiveness of the adrenal cortex to ACTH as well.

d. *Acutely Hypophysectomized Dogs Receiving a Constant Intravenous Infusion of ACTH during the Trauma and Shock Periods.* Two dogs were studied under these circumstances. One dog showed a slight increase in corticoid output during shock. The other dog showed no change in corticoid output from the post-trauma level.

Group 5: Splanchnicectomized, Partially Sympathectomized Dogs. Three dogs were studied in this group, of which two had unilateral removal of the splanchnic and lumbar sympathetic chains performed acutely following the adrenal cannulation, and one had bilateral removal 2 weeks prior to cannulation. All 3 animals showed marked increases in adrenal cortical secretion when hemorrhagic shock was superimposed on the operative trauma. This suggests that the adrenal medulla does not participate in the enhancement of adrenal cortical secretion in shock.

SUMMARY AND CONCLUSIONS

1. Operative trauma produces a marked increase in the output of 17-hydroxycorticosteroids in the adrenal venous blood of the dog over that seen in the convalescent animal. The secretion of ACTH from the pituitary in the convalescent animal is apparently intermittent and of a low order

2. The adrenal cortex is capable of maintaining high levels of corticoid secretion in severe shock in spite of markedly reduced adrenal blood flow When the mean blood pressure is reduced below 35 mm. Hg the adrenal blood flow may become so low that the minute corticoid output is reduced. The adrenal is still capable of responding to transfusion with an immediate increase in corticoid output, however

3 In some instances of hemorrhagic shock there is a marked increase in corticoid output above that seen with operative trauma alone. This usually occurs just after the bleeding has been stopped, while the shock is still profound, and continues during the transfusion of the lost blood and in the immediate post-transfusion period The effect is usually gone within 15 minutes after the blood has been replaced. This appears in part to be due to an increased ACTH release accompanying the shock, but there is some evidence to suggest that there may also be an increased adrenal response to ACTH under these circumstances This effect is not noted in the absence of ACTH. The integrity of the nerve supply to the adrenal medulla is not necessary to this response.

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blood pressure was recorded at five minute intervals. Each animal was autopsied. Particular attention was paid to (1) the completeness of the ligation, (2) the presence or absence of collateral venous pathways between the portal and systemic circulation, (3) the presence and amount of free fluid in the peritoneal cavity, and (4) the extent of ecchymosis and gross hemorrhagic infarction in the bowel wall and pancreas. The adrenal glands were carefully examined.

Results

In Table 1 are summarized the data on the animals having shock produced by ligation of the portal vein.

The results indicated that the shortest mean survival time of 95.6 minutes was in group II-B in which the increase of circulating corticoids was produced by giving ACTH intravenously. This figure was statistically significant as compared to the control animals and suggests that ACTH therapy was harmful in hemorrhagic shock of this type. There was so much variation

Table 1. Summary of Results Following Ligation of the Portal Vein in Dogs

GROUP	NO OF DOGS	MEAN SURVIVAL TIME (MIN.)	RANGE OF SURVIVAL TIME (MIN.)	P VALUE	PER CENT CHANGE IN SURVIVAL TIME
I Decreased Corticoids					
A. Adrenalectomized	6	143.5	55 to 271	>0.5	
B. Medically adrenalectomized	8	155 5	62 to 367	>0.5	
Total	14	150 3			+9.6
II. Increased Corticoids					
A Treated with Cortisone					
1. Cortisone I M.	8	129 8	88 to 155	>0 5	
2. Hydrocortisone I V.	6	110 8	88 to 148	>0.5	
3. Hydrocortisone I.A.	4	116 7	85 to 152	>0.5	
Total	18	120 6			−12 4
B Treated with ACTH I V.	8	95 6	62 to 120	<0.05	−30 3
III. Control	10	137 1	72 to 223		100.0

in the other groups that statistical analysis is not significant; however, it will be noted that the average survival time in the other groups is so similar to the survival time of the controls that one could not be impressed that corticoid therapy had exerted any influence on survival time.

The data further indicated that there was no correlation between the survival time and the weight of the animals. An autopsy was performed immediately following the death of each dog. The findings were so similar that they presented a fairly characteristic picture. The entire gastro-intestinal tract, including the stomach and the descending colon, was of a dark brownish red color. The intestinal wall was markedly thickened and edematous. The lumen contained small amounts of hemorrhagic fluid. The pancreas exhibited a marked hemorrhagic infarction with perivascular hemorrhage. As a rule this picture was more pronounced in these animals which had had a shorter survival time, and was true regardless of their adrenal cortical state. The kidneys, liver, lungs, and myocardium appeared anemic.

undergoing portal ligation was increased by 100 per cent as compared to portal ligation without splenectomy.

Knowing the difficulties connected with production of uniform shock in rats, and after numerous unsatisfactory efforts to reproduce the results of previously described procedures, a new method was developed. One femoral artery and vein were transected, and free bleeding allowed until spontaneous hemostasis took place. This procedure in our hands has been (1) simple, (2) has caused a fairly constant and comparable blood loss, and (3) has produced a fairly constant mortality in control animals. The blood loss and the mortality percentage were used as the criteria of the effect on hemorrhagic shock in rats.

LIGATION OF PORTAL VEIN IN DOGS

In this group of experiments fifty mongrel dogs of both sexes were used and divided, according to their adrenal cortical state, into three groups:

GROUP	PREPARATION	NO. OF DOGS
I	Decreased circulating corticoids	
	A Adrenalectomized	6 male
	B. "Medically adrenalectomized"	8
II	Increased circulating corticoids	
	A Cortisone treated	
	1 Cortisone intramuscularly	6
	2 Hydrocortisone intravenously	6
	3 Hydrocortisone intra-arterially	4
	B ACTH intravenously	8
III	Control animals	10

Methods

To produce decreased circulating corticoid levels in group I-A, six male dogs were adrenalectomized bilaterally Under sterile conditions both adrenals were removed in a *one stage* operation Special care was taken to remove the gland without fragmentation After convalescence of five to eight weeks, the dogs were used for the final experiment.

[illegible] " produced by giving cortisone [illegible], for fourteen consecutive days [illegible] hours before the animal was subjected to portal vein occlusion

[illegible] dose of 4 mg per kilo- [illegible] s given four hours prior [illegible]

Animals in group II-A_2 received hydrocortisone free alcohol intravenously. The infusion was started one hour preoperatively and the dose was calculated as 2 mg of hydrocortisone free alcohol per kilogram of body weight.

Animals in Group II-A_3 received the hydrocortisone free alcohol solution intra-arterially into the superior mesenteric artery at the time of operation, as a single dose of 1 mg per kilogram of body weight

In Group II-B, ACTH was given intravenously, 2 units per kilogram of body weight was the dose used

Group III constituted control animals which were paired with certain treated animals, throughout the entire experiment. They did not receive any medication except the anesthetic agent, before exposure to shock

The portal vein occlusion, preceded by splenectomy, was achieved by simple ligation at operation.

Two ligatures were placed around the portal vein proximal to the pancreaticoduodenal

four hours preoperatively and the other half was given immediately after the bleeding had ceased. Group V-H control animals were treated in a similar way except that they received 0.2 ml. of saline intramuscularly.

The section of the femoral artery and vein was performed in the following manner: Before an experiment was started each rat was weighed and identified. The experimental animals and their controls were operated upon the same day at approximately fifteen [illegible] the animals from both groups. The rats were lightly anesthe- [illegible]

[illegible] by intermittent sponging with a preweighed gauze sponge. [illegible] spontaneous hemostasis occurred. The blood loss was determined by weighing the saturated sponge on an analytical balance immediately after the bleeding had stopped.

The mortality was recorded and ascribed to the hemorrhage if the animal died within the first 24 hours postoperatively. The dead animals were autopsied.

Results

The data on this study are contained in Table 2.

Decreased Circulating Corticoids. In the twenty adrenalectomized rats of group IV-A there was an average blood loss of 2.57 per cent of body weight and a mortality rate of 60 per cent, compared to a blood loss of 2.77 per cent of the body weight and a mortality rate of 35 per cent in the twenty sham adrenalectomized rats.

Group IV-C shows a marked difference in weight as compared to their control, group IV-D; their average weight after cortisone therapy was 123.4 gm. compared to 164.9 gm. for the controls. Before the cortisone treatment was started, both groups weighed the same, namely 154.2 gm. Therefore the cortisone in this dose appeared toxic. The average blood loss in group IV-C was 2.41 per cent of body weight as compared to a blood loss of 1.96 per cent in the control group. The difference between both groups was statistically significant, as indicated by a P value which was smaller than 0.05. The mortality was the same in both groups, namely 20 per cent.

When the experiment was repeated (group IV-C_1) using only 1.0 mg. of cortisone to produce the adrenal suppression, the average blood loss was 2.63 per cent as compared to the control group, which lost 2.77 per cent. This shows a reversal of the previous results and may be closer to the true picture since there was no weight loss in either group during the cortisone treatment period. The mortality again in both groups remained equal at 20 per cent.

Increased Circulating Corticoids. In the twenty rats receiving a heavy dose of cortisone (group V-A) the average blood loss was 1.91 per cent of body weight, and the mortality was 20 per cent. In the twenty control rats [illegible] blood loss was 1.99 per cent of [illegible] per cent. The difference in blood [illegible]

The results indicated that there was no difference between the group treated with a low dose of cortisone (group V-C) and its control (V-D) receiving no cortisone. For example, the blood loss in group V-C was 1.52 per cent of body weight as compared to 1.51 per cent in the control of group V-D. The P value was well above 0.5.

Groups V-E and V-F contained ten rats each, nutritionally depleted; group V-E received a short course of cortisone therapy preceding hemorrhage. The data indicates that the cortisone treated group has a larger blood

The adrenals of the "medically adrenalectomized" group (given cortisone) appeared to be definitely smaller than those of any other group. No other macroscopic changes in the adrenals were noted.

SECTION OF THE FEMORAL ARTERY AND VEIN IN RATS

This experiment contains 180 white female rats (Holtzman strain) subjected to hemorrhage by severing the femoral artery and vein; according to their adrenal cortical state they were divided into two main groups, with controls for each subgroup.

GROUP	PREPARATION	NO. OF RATS
IV	Decreased circulating corticoids	
	A. Adrenalectomized	20
	B. Sham adrenalectomized	20
	C "Medically adrenalectomized," high dose	10
	D. Controls	10
	C_1. "Medically adrenalectomized," low dose	10
	D_1 Controls	10
V	Increased circulating corticoids	
	A Cortisone treated high dose	20
	B. Controls	20
	C. Cortisone treated low dose	10
	D Controls	10
	E Cortisone treated, nutritionally depleted	10
	F. Nutritionally depleted controls	10
	G Corticotropin treated	10
	H. Controls	10

Methods

Before subjecting the rats to hemorrhage, their adrenal cortical function was altered as follows

In group IV-A, a one stage bilateral adrenalectomy was performed on rats under intraperitoneal pentobarbital anesthesia The postoperative management consisted of 0 9 per cent NaCl solution as drinking water ad libitum No other medications were used.

In group IV-B, "sham" adrenalectomy consisted of anesthesia, incision, and manipulations of viscera as in group IV-A, but without actual removal of the adrenals. A period of thirty days was allowed for complete recovery before the animals were used for the final experiment

The rats of group IV-C were prepared by administration of cortisone acetate given intramuscularly in a dosage of 5 mg per rat per day for fourteen consecutive days This resulted in marked weight loss A repeat experiment, IV-C_1, was then done using cortisone acetate 1 mg per rat daily intramuscularly for fourteen days The control animals (groups IV-D and IV-D_1) received 0 2 ml of saline intramuscularly on corresponding days. A seventy-two hour period was allowed to elapse between the last dose of cortisone and the hemorrhage

In group V-A, cortisone was given intramuscularly in a dose of 5 mg per rat per day for three consecutive days The last dose was given four hours before the rats were subjected to hemorrhage Control animals in group V-B received 0 2 ml. of saline intramuscularly on corresponding days

Groups V-C and V-D were treated in a manner similar to the preceding two groups except that the cortisone dose (in group V-C) was reduced to one-tenth of the dose in group V-A

Group V-E, and its control animals in group V-F, were nutritionally depleted utilizing a low protein diet. After a period of six weeks on this diet, group V-E was given cortisone acetate 5 mg per rat per day on three successive days The last dose was given four hours before the hemorrhage was instituted Group V-F was given saline at the same time as group V-E received the cortisone

Group V-G received ACTH 2 5 units intramuscularly, one-half of which was given

four hours preoperatively and the other half was given immediately after the bleeding had ceased. Group V-H control animals were treated in a similar way except that they received 0 2 ml. of saline intramuscularly.

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When the experiment was repeated (group IV-C_1) using only 1.0 mg. of cortisone to produce the adrenal suppression, the average blood loss was 2.63 per cent as compared to the control group, which lost 2.77 per cent. This shows a reversal of the previous results and may be closer to the true picture since there was no weight loss in either group during the cortisone treatment period. The mortality again in both groups remained equal at 20 per cent.

Increased Circulating Corticoids. In the twenty rats receiving a heavy dose of cortisone (group V-A) the average blood loss was 1.91 per cent of body weight, and the mortality was 20 per cent. In the twenty control rats not receiving cortisone (group V-B) the blood loss was 1.99 per cent of body weight and the mortality rate was 5 per cent. The difference in blood loss was insignificant.

The results indicated that there was no difference between the group treated with a low dose of cortisone (group V-C) and its control (V-D) receiving no cortisone. For example, the blood loss in group V-C was 1.52 per cent of body weight as compared to 1.51 per cent in the control of group V-D. The P value was well above 0 5.

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	B. Sham adrenalectomized	20
	C "Medically adrenalectomized," high dose	10
	D. Controls	10
	C_1 *"Medically adrenalectomized," low dose*	10
	D_1. Controls	10
V	Increased circulating corticoids	
	A Cortisone treated high dose	20
	B. Controls	20
	C. Cortisone treated low dose	10
	D Controls	10
	E Cortisone treated, nutritionally depleted	10
	F Nutritionally depleted controls	*10*
	G. Corticotropin treated	10
	H. Controls	10

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Group V-G received ACTH 2 5 units intramuscularly, one-half of which was given

loss (1.89 per cent of body weight) and a higher mortality (40 per cent) as compared to their control animals in which the blood loss was 1.60 per cent of body weight and the mortality rate 10 per cent.

In the rats treated with ACTH (group V-G) the blood loss was 2.25 per cent of the body weight and the mortality rate 20 per cent. These figures were higher than in the controls (V-H) in which the blood loss was 2.03 per cent of the body weight and the mortality rate was zero.

The data of this study indicated that decreased circulating corticoids caused a slightly higher mortality and an insignificantly smaller blood loss as compared to their control animals. In the increased circulating corticoid group the mortality was markedly higher and the blood loss was insignificantly greater when compared to the control group.

It was noted further that the individual variations in the amount of blood lost between different animals were greater than the difference of means between the experimental groups and their controls.

The autopsy on rats which expired shortly after the hemorrhage revealed the gastro-intestinal tract, including the duodenal mucosa, and the kidneys, liver, lungs and myocardium, to be anemic. No other gross changes were noticeable. The adrenals in the medically adrenalectomized group definitely appeared smaller; however, no measurements were obtained.

DISCUSSION

Since the species difference in response to trauma is very important it was felt that utilization of dogs and rats for a single investigation would be of benefit. Low corticoid levels were obtained by two methods: (1) surgical adrenalectomy and (2) "medical adrenalectomy" by giving cortisone. The adrenalectomized group, by definition, in absence of ectopic adrenal cortical tissues should have a decreased corticoid level. The basis for adrenal suppression was found in Ingle's and Kendall's work.[3] They demonstrated that the administration of large doses of exogenous corticoids resulted in atrophy of the adrenal cortex of rats. The shortest time interval necessary for production of adrenal atrophy was found to be between five days[4] and ten days.[5] The time necessary for recovery of the atrophied adrenal cortex after exogenous suppression varied from four days[5] in rats to two months or more in human beings.[6] It was necessary to establish the optimum time for shock procedure, at which point there was the lowest endogenous corticoid level, and at the same time very little, if any, carry-over effects from the previous exogenous cortisone administration. This optimum period is thought to be between forty-eight and seventy-two hours after discontinuation of cortisone.[7] Therefore, it was felt safe to assume that the "medical adrenalectomy" had produced decreased corticoid levels at the time the shock procedures were applied in these experiments.

The increased circulating corticoid levels at the time of shock were created by exogenous cortisone, hydrocortisone, and ACTH administration. The observation that cortisone and ACTH produce increased amounts of 11-oxysteroid levels in the blood from the adrenal vein in dogs and men served as evidence for calling the animals so treated the increased circulating corticoid group.[8] Again it was important to determine the time, after intramuscular cortisone injection, at which the circulating corticoids would reach their peak, as the optimum time for experimental shock. This was found to be within 48 hours.[7] Therefore, a three day cortisone pretreatment

Table 2. Summary of Results Following Section of Femoral Artery and Vein in Rats

GROUP	NO OF RATS	MEAN WT GM	MEAN BLOOD LOSS IN GM.	BLOOD LOSS % OF BODY WEIGHT	RANGE	MORTALITY %	P VALUE FOR BLOOD LOSS
IV-A Adrenalectomized	20	216	5 587	2 57	1 67-3 36	60	
IV-B Sham adrenalectomized	20	223	6 188	2 77	1 88-3 14	35	0 05
IV-C "Medically adrenalectomized," high dose	10	123 4	2 986	2 41	1 59-3 39	20	
IV-D Controls	10	164 9	3 194	1 96	1 68-2 76	20	0 05
IV-C$_1$ "Medically adrenalectomized," low dose	10	202	5 331	2 63	2 13-3 04	20	
IV-D$_1$ Controls	10	218 3	6 067	2 77	2 55-3 17	20	0 2
V-A Cortisone treated, 5 mg	20	172 9	3 428	1 91	1 18-3 05	20	
V-B Controls	20	175 3	3 455	1 99	1 11-3 22	5	0 5
V-C Cortisone treated, 0 5 mg	10	187 2	2 856	1 52	1 11-2 09	0	
V-D Controls	10	194 3	2 937	1 51	1 12-2 32	0	
V-E. Depleted, cortisone treated	10	167	3 132	1 89	1 10-3 35	10	
V-F. Depleted controls	10	172 4	2 764	1 60	1 83-2 14	10	0 2
V-G. ACTH treated	10	200 9	4 241	2 25	1 16-3 61	20	
V-H Controls	10	223 7	4 309	2 03	1 09-2 76	0	0 5

STEROID PRODUCTION BY INCUBATED HUMAN ADRENAL TISSUE*

D. Y. COOPER, J. M. ROBERTS, AND J. C. TOUCHSTONE**

The majority of studies of adrenal physiology have employed techniques designed to measure the steroids excreted in the urine. A few studies have been made to determine the steroids which are present in the peripheral blood and in adrenal venous blood. Pincus[1] and his group have perfused human adrenal glands, obtained freshly at operation, with a saline solution and extracted the steroids from the perfusate. Under these conditions they only obtained small quantities of compounds F (hydrocortisone) and B (corticosterone). Another approach has been used by Brady,[2,3] in which dog glands are sliced immediately after removal and incubated in plasma taken from the same animal. The steroids were extracted from the homogenized gland and the medium. Compounds F, B, S (17-hydroxy-11-desoxycorticosterone), A (11-dehydrocorticosterone) and possibly E (cortisone) have been partially identified with this method. The status of knowledge of adrenal metabolites has recently been summarized by Hechter.[4]

At the Hospital of the University of Pennsylvania glands were obtained from individuals subjected to adrenalectomy for severe progressive hypertension and, in certain instances, from patients from whom these glands were removed as palliative treatment in advanced cancer of the breast and prostate. It has been found possible to incubate these glands in a manner similar to that of Brady, to extract the homogenized gland and to separate the steroids produced by paper chromatographic techniques.

METHODS

Adrenal glands were obtained at operation from patients with hypertension and patients with carcinoma of the breast or prostate. The glands were immediately placed in a flask cooled in an ice water bath, transferred to a cold room at 4°C., and freed of the periadrenal fat and capsule. The wet weight of the gland was determined prior to sectioning. Sections of the gland were taken for pathologic examination and for total nitrogen determination. First, the portion of gland to be incubated was cut into thin slices with a Stadie-Riggs tissue slicer. The slices (0.4 to 1.0 gm. of tissue) were then transferred into a chilled 125 ml. Erlenmeyer flask and reweighed. Twelve to 15 cc. of heparinized plasma was then added to each flask together with 50,000 units of penicillin and 0 1 gm. of streptomycin. This flask was stoppered with a two way cork stopper connected to a gas inlet and outlet tube. The Erlenmeyer flask was attached then to a Warburg manometer support and immersed in a water bath at 37.5°C. The flask was connected to a gas mixture of 95 per cent O_2–5 per cent CO_2 and shaken at a rate of 50 to 60

* From the Harrison Department of Surgical Research, Schools of Medicine, University of Pennsylvania, and the Endocrine Section, Wm. Pepper Laboratory for Clinical Medicine, Hospital of the University of Pennsylvania, Philadelphia. This study was supported
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Doctors H. A. Zintel, W. T. Fitts, Jr. and Paul Leberman for supplying us with human adrenal tissue, and to Doctor Otto Rosenthal for his helpful advice during the progress of this work.

schedule was chosen The prolonged exogenous cortisone pretreatment, i e, over a period of four or more days, may result in adrenal cortical suppression, and therefore would deprive the animals under extreme stress, such as shock, of additional endogenous corticoid formation. The intra-arterial route of hydrocortisone free alcohol was employed to carry the steroid directly to the site of traumatized tissue without diluting it first in the systemic circulation. ACTH given intravenously produces an immediate adrenal cortical response with sudden rise of circulating corticoids in the adrenal venous blood.[8] Therefore, when the intravenous route was utilized in dogs, corticotropin was given one-half hour prior to portal occlusions and again twenty minutes thereafter.

SUMMARY

The variations of the survival time in dogs and of the blood loss in rats during these experiments were too great to allow one to draw definite conclusions on the influence of increased or decreased circulating corticoid levels on the tolerance to this type of shock. The decreased circulating corticoids did not appreciably influence the survival time in dogs or definitely alter the blood loss or mortality rate in rats. The increased circulating corticoids appeared to have no beneficial influence in prolonging the survival time after portal ligation in dogs and caused a definitely increased mortality rate in rats subjected to hemorrhagic shock. Accordingly, the various trends in the data of the experiments did not reveal any evidence that corticoid therapy exerted any beneficial effect on the two types of blood loss and hemorrhagic shock utilized in these experiments. The slight amount of statistically significant difference noted indicated that the steroids may actually be harmful in shock.

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Anoxia Experiments

The importance of optimal oxygenation for steroid formation was tested in an experiment in which one flask containing the adrenal slices was allowed to stand at room temperature for a period of 45 minutes before

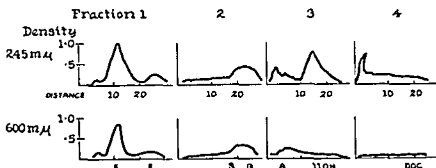


Fig. 1. Typical chromatographic pattern of steroids produced by adrenal incubates.

oxygenation and incubation for 24 hours in the usual manner. There was a sevenfold decrease in F production while decreases in the amount of 11-β-hydroxyandrostenedione were not as large.

Control Experiments

In order to show that the steroids obtained were produced by the adrenal tissue during the period of incubation, control extraction of non-incubated tissue plus plasma was done. In four separate experiments the average amount of compound F that could be obtained was 20 γ per gram of gland plus 15 cc. of plasma. This is in contrast to a rough average of 500 γ of compound F per gram after incubation of the gland for 24 hours.

Stimulation by ACTH

The effect of the addition of 10 units of corticotropin to the incubate is shown in Figure 2. A 70 per cent increase in F production is evident. The

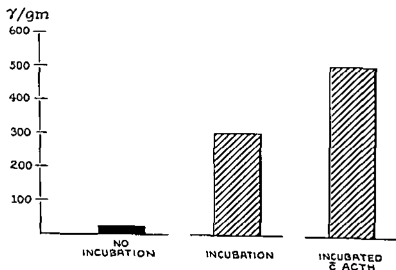


Fig 2. Effect of incubation and addition of corticotropin on compound F production by adrenal incubates

oscillations per minute through an amplitude of 4 to 5 cm. The gas mixture was passed as a slow current through the flasks for the entire time of incubation.

The *p*H of the serum samples averaged *p*H 7.3 and did not change significantly during incubation, so no additional buffer was added. The time interval between removal of the gland and the start of the experiment was seldom more than 45 minutes.

In the ACTH experiments 10 units of corticotrophic hormone were added to each flask.

After completion of the incubation the tissue and medium was quantitatively transferred to an all glass homogenizer of the Potter Elvehjem type and homogenized Five volumes of acetone were added to the homogenate to precipitate the proteins The precipitate was removed by filtration and washed well with additional acetone before the acetone was removed in vacuo in a water bath not exceeding 45°C. The aqueous phase remaining was extracted twice with 50 ml. of ethyl acetate and twice with 25 ml. volumes of chloroform. The organic extracts were combined and washed successively twice with 0.1 N sodium hydroxide, 25 ml. each and twice with 50 ml. volumes of water The organic phase was evaporated to dryness in vacuo in a water bath whose temperature did not exceed 45°C. The residue so obtained was subjected to partition paper chromatography as described below.

It was necessary to determine the optimal amount of the residue that was placed at the starting line of each 0.5 inch strip in order to get a good chromatographic separation. This amount depended upon the size of the incubated tissue and varied from one-third to one-twelfth of the total extract

In three experiments 50 micrograms of hydrocortisone and cortisone were added to 15 ml samples of plasma and extracted as described above. Recoveries were 95 to 105 per cent of that amount added.

Methods of Separation. The adrenal extracts were divided into five main fractions designed to separate six active cortical steroids. Fraction I consisted of material found on a paper chromatogram run in a toluene-propylene glycol system after 13 cc. of effluent had been collected per 0 5 inch width of strip This fraction contained any compound F or E present Fraction II represented that strip on which the residue from evaporation of the effluent from fraction I had been placed and developed until 3 cc. of effluent per 0.5 inch strip was collected The effluent from fraction II was evaporated to dryness and placed on a third strip and developed in a methylcyclohexane-propylene glycol system until 17 cc of effluent per 0 5 inch strip was collected. The effluent from fraction III was evaporated to dryness and placed on a strip in the methylcyclohexane system and developed until 3 cc of the effluent was collected. This fraction IV contained DOC and other unidentified materials The effluent of this strip was run to the end in the methylcyclohexane-propylene glycol system to give fraction V.

The strips, after drying in air, were read at 245 mμ in a Beckman spectrophotometer using an adaptor as described by Tennant et al.[5] The optical density was noted in relation to distance from the starting line on the strip. The strips were finally sprayed with blue tetrazolium (dianisole bisdiphenyl tetrazolium chloride) and read in a similar manner at 600 mμ. These density values were plotted against distance on the strip to give a typical record as shown in Figure 1.

The results so far obtained reveal that in terms of production of steroid per unit weight of tissue compound F was the major product found in the incubates and was invariably present. Material with the properties of compound E was found in all but two of 21 incubated glands. Compounds B and S were found in a smaller proportion of the incubates. By our present methods, there is no satisfactory way of separating these two compounds. Consequently, only the combined total is reported for amounts of these compounds.

11-β-Hydroxyandrostenedione appeared consistently in the incubate in quantities second only to that of compound F.

SUMMARY

A study of incubated human adrenals has been made. It has been possible to isolate and partially identify the steroid compounds F, B, S and 11-β-hydroxyandrostenedione. Evidence has also been obtained for the presence of compound E. The production of these cortical hormones is stimulated by the addition of corticotropin to the incubation mixture. When the tissues were incubated in the absence of oxygen the steroid production is negligible. The production of steroids has been shown to continue for at least 24 hours.

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ADRENOCORTICAL RESERVE IN DEBILITATED SURGICAL PATIENTS*

EUGENE JABBOUR AND JAMES D. HARDY

There currently exists a considerable interest in the possible usefulness of ACTH and cortisone in debilitated patients who require surgery. Hume and Moore[1] and Cole and his associates[2] have reported favorably concerning the empiric use of these agents in individuals who did not exhibit clinical evidence of adrenocortical insufficiency. The purpose of the present study has been to attempt to identify debilitated patients with a limited adrenocortical reserve and to define the incidence of such cases.

METHODS AND PROCEDURE

The subjects were chosen because of marked weight loss due to ad-

* From the Department of Surgery and Surgical Laboratories, Medical College of the University of Tennessee, and The John Gaston Hospital, Memphis. This work was done under Army Contract No. DA-49-007-MD-296.

amount of material which is thought to represent E and other compounds is also increased when ACTH is added. Similar increases were found in 85 per cent of the glands studied. On the other hand, the increase of 11-β-hydroxyandrostenedione was not significant.

Effect of Time on Steroid Production

Various sections of the same gland were incubated for increasing lengths of time. In Figure 3 the production of 17-hydroxycorticosterone is plotted against time These experiments indicate that steroid production by adrenal slices under these conditions is a function of time and continues over a 24

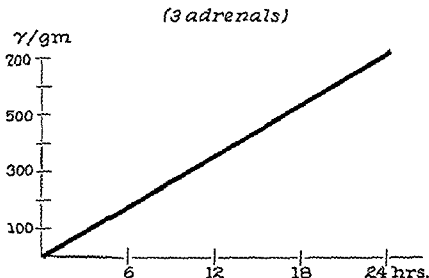


Fig 3 Relationship of time to compound F production by adrenal incubates

hour period. A similar increase in production was observed for each of the compounds studied.

RESULTS

Chromatographic Pattern. In Figure 1 curves representing both the ultraviolet absorption of 245 mμ and the absorption at 600 mμ after spraying the same strips with blue tetrazolium are shown

In fraction I the peaks shown are consistent with chromatographic properties of compound F (hydrocortisone) and compound E (cortisone) in the same system. However, several other compounds have been separated from the zone which represents E on the chromatographic strip.

Fraction II contains compounds consistent in properties with compound B (corticosterone) and compound S (17-hydroxy-11-desoxycorticosterone). In the third fraction compound A would ordinarily appear. No convincing evidence for its presence has been obtained in these experiments, but a compound partially identified as 11-β-hydroxyandrostenedione is consistently found. In fraction IV, desoxycorticosterone should appear. In none of the incubates is this compound indicated. Fraction V, not shown in the figure, contains no definite peaks but shows large quantities of material reacting with blue tetrazolium.

have been in the hospital for several days, before reporting the corticoid data in detail.

Adrenocortical Response in *Subjects Awaiting Elective Herniorrhaphy.* To confirm and to buttress the data reported by Renold and his associates[3] in 41 normal subjects, five additional "normal" subjects have been studied by us to date (Fig. 1). Renold and his co-workers found that the intravenous infusion of 20 units of ACTH over a period of eight hours produced a mean increase of 6.3 mg. in the excretion of 17-ketosteroids during the 24-hour period of the infusion as compared with the preceding control day. The mean increase in ten persons with Addison's disease was 0.4 mg., and following bilateral adrenalectomy the mean increase was 0.1 mg. in eight

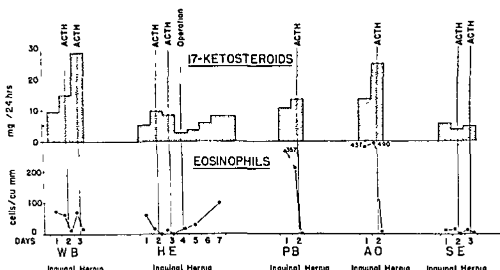


Fig. 1. Note that the intravenous ACTH produced an increase in 17-ketosteroid excretion in four of the five "normal" subjects
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to the absence of a rise in 17-ketosteroid excr
subject was evidence that there was at least some degree of adrenocortical activity as was, indeed, his uneventful tolerance for operation The excretion of 17-ketosteroids is *not commonly elevated following operation,* exhibited above by H.E. Thus, operation and ACTH appear to produce somewhat different response in adrenocortical activity.

patients. In normal subjects the total eosinophil count was almost without exception reduced by more than 50 per cent of the pre-infusion level.

The intravenous infusion of 20 units of ACTH in our five "normal" subjects produced the characteristic response in 17-ketosteroid excretion in all except one individual. This patient (S.E , a 68 year old male) exhibited a control total eosinophil count of almost zero, indicating that he was under stress of some type, whether emotional or nutritional. He was not febrile. Yet, subject H.E. (age 48) also had a low pre-infusion count but did exhibit a rise in the excretion of both corticoids and 17-ketosteroids. The failure of ACTH to produce a rise in 17-ketosteroid excretion in patient S.E. may have reflected in part the decreased adrenocortical activity often observed in elderly individuals. Nevertheless, the excretion of corticoids also failed to

vanced malignancy or, in one instance, because of severe debility due to a ruptured appendix with widespread peritonitis in an elderly female. The intravenous ACTH test of Renold and his associates[3] was employed to measure adrenocortical reserve. During a 24-hour control period the total eosinophil count (eosin-acetone technique) was determined and the urine collected for 17-ketosteroid[4] and corticoid[5] analyses. On the following day the patient received intravenously over an 8-hour period 1000 cc. of glucose or saline solution containing 20 units of ACTH (Armour, Lot No. M62809 c) Total eosinophil counts were made before and after the infusion and, again, a complete 24-hour urine collection was made In general, the procedure used during the second period was again employed during a third 24-hour period. Finally, in a number of subjects a fourth day was employed as a second control period. The response of several of these individuals to operation was also studied

Table 1.

	PATIENTS	AGE	SEX	DIAGNOSIS	WEIGHT LOSS (LBS.)
1	W B	60	M	Inguinal hernia	None
2	H E	48	M	Inguinal hernia	None
3	P B	40	M	Inguinal hernia	None
4	A O	28	M	Inguinal hernia	None
5	S E	68	M	Inguinal hernia	None
6	D J	61	M	Carcinoma of stomach	50
7	W H	56	M	Carcinoma of stomach	30
8	G J	62	M	Carcinoma of stomach	35
9	V J	60	F	Carcinoma of stomach	40
10	R McK	56	M	Carcinoma of stomach	35
11	A P	70	F	Carcinoma of esophagus	25
12	G P	62	M	Carcinoma of esophagus	20
13	N S	50	F	Carcinoma of esophagus	30
14	W D	55	M	Carcinoma of esophagus	40
15	W T	55	M	Carcinoma of pancreas	25
16	J S	63	F	Carcinoma undetermined site	35
17	A C	65	F	Ruptured appendix	10
18	W M	72	M	Carcinoma prostate	40
19	J H	59	M	Carcinoma of stomach	35

Normal values for 17-ketosteroid excretion in our laboratory range from 5 to 15 mg in females and from 7 to 21 mg in males It is, of course, not easy to set definite "normal" limits for the total eosinophil counts However, rarely is the initial count below 50 cells per cubic millimeter in the absence of some type of stress stimulus and control counts which exceed 400 cells per cubic millimeter are definitely unusual in the presence of actively functioning adrenal cortices.

RESULTS AND DISCUSSION

The incidental data concerning the patients studied are given in Table 1, and the total eosinophil counts and the urinary excretion of 17-ketosteroids in Figures 1-4.

The urinary excretion of corticoids was also measured. However, the control values in two of the "normal" subjects, as measured in the urine excreted shortly after admission to the hospital for a major operation, were elevated; we therefore wish to study additional normal subjects after they

have been in the hospital for several days, before reporting the corticoid data in detail.

Adrenocortical Response in Subjects Awaiting Elective Herniorrhaphy. To confirm and to buttress the data reported by Renold and his associates[3] in 41 normal subjects, five additional "normal" subjects have been studied by us to date (Fig. 1). Renold and his co-workers found that the intravenous infusion of 20 units of ACTH over a period of eight hours produced a mean increase of 6.3 mg. in the excretion of 17-ketosteroids during the 24-hour period of the infusion as compared with the preceding control day. The mean increase in ten persons with Addison's disease was 0.4 mg., and following bilateral adrenalectomy the mean increase was 0.1 mg. in eight

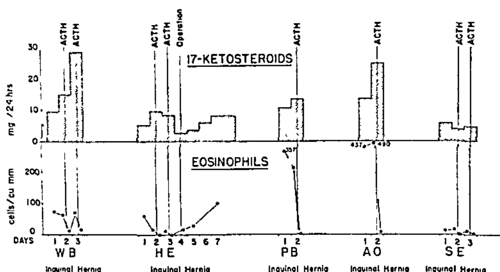


Fig 1. Note that the intravenous ACTH produced an increase in 17-ketosteroid excretion in four of the five "normal" subjects awaiting elective herniorrhaphy. The fifth subject (S E) was the oldest of this group (see Table 1), and this may have contributed to the absence of a rise in 17-ketosteroid excretion The low total eosinophil count in this subject was evidence that there was at least some degree of adrenocortical activity as was, indeed, his uneventful tolerance for operation The excretion of 17-ketosteroids is not commonly elevated following operation, exhibited above by H.E. Thus, operation and ACTH appear to produce somewhat different response in adrenocortical activity.

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increase. The total eosinophil count, already at zero, did indicate a degree of adrenocortical activity, and he underwent operation without event. Had 40 units of ACTH been used, an increase might have occurred (see G.J., Fig. 2).

Adrenocortical Reserve in Patients with Carcinoma of the Stomach. For convenience, the five patients who had advanced gastric carcinoma are grouped together (Fig. 2). Three patients (D.J., W.H., and R.McK.) exhibited a relatively "normal" response, but the other two subjects are of particular interest. Patient G.J. exhibited a relatively mild response to both intravenous ACTH (20 units) and operation (jejunostomy), but responded to the larger dose of 40 units of ACTH with a sharp rise in excretion of corticoids and 17-ketosteroids and a fall in the total eosinophil count. Therefore it would appear that the pituitary stimulus, rather than the functional

Adrenocortical Reserve in Patients with Carcinoma of the Stomach
The Intravenous ACTH Test

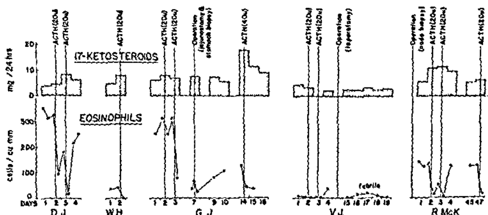


Fig 2 Two of these patients, G J, and V J, exhibited little response to intravenous ACTH Note the absence of a significant fall in the total eosinophil count following ACTH

tion. Both patients had uneventful postoperative courses, indicating at least a modicum of adrenocortical activity.

reserve of the adrenal cortices, was deficient—though a decreased sensitivity of the adrenal cortex to ACTH cannot be excluded. Patient V.J. exhibited no rise in either corticoid or 17-ketosteroid excretion following ACTH. Likewise, following exploratory laparotomy and gastrojejunostomy no rise in corticoid excretion occurred.

In spite of these findings this patient had a relatively uneventful postoperative course, indicating that at least some degree of adrenocortical function was present.

Adrenocortical Reserve in Patients with Esophageal Carcinoma (Fig. 3). All four subjects exhibited an increase in 17-ketosteroid excretion and a decrease in the total eosinophil count, though the latter was already near zero in three subjects owing to the starvation and other stress associated with their disease.

Adrenocortical Response in Debilitated Patients with Various Other Dis-

withstood exploratory laparotomy. Subject J.S., who had ...
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... the
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Adrenocortical Reserve in Patients with Esophageal Carcinoma
The Intravenous ACTH Test

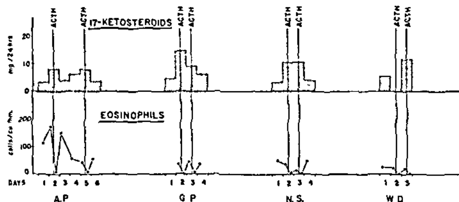
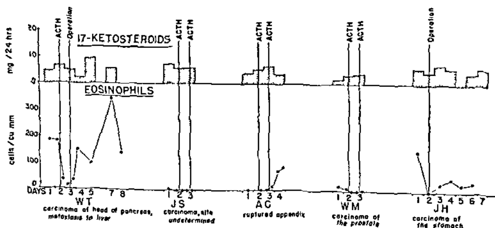


Fig. ... th esophageal carcinoma responded well to ... patients had low control eosinophil counts, ...

Adrenocortical Reserve in Various Debilitated Patients
The Intravenous ACTH Test



... following operation, ...

disease, and there was a definite increase in the excretion of corticoids. Patients A.C and W.M both had an increase in the excretion of both corticoids and 17-ketosteroids, the total eosinophil count of zero precluded further interpretation based upon changes in these values. Patient J.H. exhibited no increase in either corticoid or 17-ketosteroid excretion following operation, but while there was little evidence of an increase in adrenocortical activity, he did withstand the surgery uneventfully.

SUMMARY AND CONCLUSIONS

The intravenous ACTH test has been used in five apparently normal and fourteen markedly debilitated patients with a view to determining the incidence of relative adrenocortical insufficiency in chronically ill surgical patients. In one 68 year old "normal" individual and in one of the chronically ill subjects there was no increase in the urinary excretion of either corticoids or 17-ketosteroids, presumably indicating a diminished adrenocortical responsiveness. However, both of these subjects had total eosinophil counts which were almost at zero during the control period (indicating at least a degree of adrenocortical activity), and both withstood operation without event A third subject, whose response to operation only was measured, exhibited no increase in the excretion of either corticoids or 17-ketosteroids. Yet, the eosinophil count fell to zero, and he underwent the operation without difficulty Thus, the total eosinophil count emerges as a useful test

It is concluded that while a diminished adrenocortical response may be observed not uncommonly in chronically ill subjects, it must be rare that these subjects cannot survive surgery without replacement therapy.

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A STUDY OF THE FREE 17-HYDROXYCORTICOIDS IN THE PERIPHERAL BLOOD OF SURGICAL PATIENTS*

RICHARD W. STEENBURG

The availability of methods for the measurement of the free 17-hydroxycorticoids in serum affords the opportunity of quantitating directly the levels of adrenal cortical hormone in the experimental animal and human subject. Such measures of the 17-hydroxycorticoids are of particular interest in view of previous demonstrations that the largest fraction of cortical steroid in the peripheral blood falls within this chemical group.[1,2] The measurement of this quantity should reflect more adequately the available steroid than does that quantity of 17-hydroxycorticoid excreted in the urine (and removed from the sites of its biologic activity), or than the 17-ketosteroid excretion.

The method of Nelson and Samuels was used for the determination of the free 17-hydroxycorticoid levels in serum.[3] In our laboratory, this method has given results in the normal range which are reproducible within 1.5 gamma per cent. Recoveries of cortisone from normal serum in ranges from 20 to 60 gamma per cent have been accurate within 10 per cent.

This chemical assay, while a reproducible one, does not measure the total steroid content of blood. Conjugates are not removed in the initial chloroform extraction. The free steroid fraction which is measured consists in large part of tetrahydro F and Compound F (hydrocortisone), of which the latter is biologically active. The biologically active fraction of this chemical quantity represents approximately 30 to 50 per cent of the whole,[4] but, since the conjugates are not active, appears to be the best available assay of the minute quantities of active cortical steroid in peripheral blood.

NORMAL ACTH RESPONSE

Using the urinary excretion rates of 17-hydroxycorticoids as an index of adrenal activity, Thorn et al.[5] have concluded that there is a maximum effective dosage of ACTH. The administration of additional quantities above this limit results in no increment in cortical steroid production. This maximum quantity is dependent upon the route and duration of administration and is slightly exceeded by the 25 unit, eight hour intravenous infusion which we have used. With this infusion, it is possible to define the levels of free 17-hydroxycorticoids in peripheral serum after maximal adrenal stimulation with ACTH in the normal subject. There is a gradual rise from normal levels to a maximum of 35 to 45 gamma per cent by the sixth hour of infusion. Doubling the rate of administration by increasing the dose of ACTH results in no appreciable alteration in the rate of response or in the peak

* From the Laboratories of Surgical Research, Peter Bent Brigham Hospital, and the Harvard Medical School, Boston, Massachusetts This work was supported by a grant from the Atomic Energy Commission to the Peter Bent Brigham Hospital, and by a grant from the United States Army, Office of the Surgeon General, to the Harvard Medical School. We also wish to acknowledge the assistance of Winthrop-Stearns, Inc., and the Upjohn Company.

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levels attained. Neither is there an apparent alteration in the response following a second infusion of drug at two day intervals.

RESPONSE TO SURGICAL OPERATIONS

These curves of the circulating steroid levels resultant upon maximal ACTH stimulation of the adrenal offer an interesting contrast to t' se observed during the course of surgical operations in the human.[6, 7] ving the onset of operation, there is a rapid rise in the blood levels 17-hydroxycorticoids to 50 to 70 gamma per cent at six or eight ho is followed by a similarly rapid fall which is at times interrupted by ary peak associated with recovery from general anesthesia. We served occasional depressed levels on the first postoperative d secondary rise in the range of 20 to 30 gamma per cent, but more there is a gradual decline to normal during the first, second, and operative days. The peak blood levels following major surgical are greater than those produced by maximal adrenal stimulation over a comparable period of time in the normal subject. It is parent that the rate of rise is greater in all save the most minor

Since there is little concrete evidence that the adrenal cortex substances other than ACTH, it would seem to be of interest to response to maximal ACTH stimulation in the presence of t' incurred with surgery.

interested in elucidating those factors associated with surgery which may initiate such a response.

EFFECT OF VARYING TYPES OF ANESTHESIA

The liver appears to be of importance in the conjugation and intermediary metabolism of the steroid hormones prior to their excretion in the urine.[9] It therefore is pertinent in the study of this phenomenon to assess the separate effects of trauma and of such chemical anesthetic agents as ether on its occurrence.

A three hour pentothal-ether anesthesia in a normal volunteer studied in this laboratory was associated with a rise in the free corticoid levels to 32 gamma per cent at the end of the anesthesia. A secondary rise to 39 gamma per cent was associated with recovery of consciousness. This rise was transient and normal levels were measured within twenty-four hours of the induction of anesthesia. Obviously the demonstration that this maximum level approximates that seen with operations of the magnitude of herniorrhaphy emphasizes the role of general anesthesia alone on the response in the free 17-hydroxycorticoids, and makes imperative the study of the response to trauma alone in the absence of these effects of general anesthesia.

Further studies on patients undergoing major operations under high spinal anesthesia have been undertaken. Previous investigators have emphasized the effect of spinal cord section on the adrenal response to trauma in a denervated extremity.[10] This work demonstrates that sensory afferents to the central nervous system must remain intact for the occurrence of the normal adrenal response to injury. It is not surprising, then, that in the patient operated upon under spinal anesthesia, no rise in corticoid levels is seen until clinical recovery of sensation is evident. Following recovery of sensation in the operated part, there is an associated rise of the circulating corticoid levels proportional to the magnitude of operation and of duration similar to that previously seen during operations under general anesthesia. Our data on the absolute quantity of this rise during major operations under spinal anesthesia are sufficiently incomplete to obviate any conclusion at this time. Others have reported levels of 17-hydroxycorticoids in the peripheral blood of patients undergoing operations with spinal anesthesia comparable to those we have reported above with general anesthesia, and greater than are seen with maximal ACTH stimulation in the normal subject.[11] These observations, while unconfirmed, suggest that the apparent defect in the intermediary metabolism of cortical steroids is a result of surgery itself apart from the effects of the anesthetic agent.

SUMMARY AND CONCLUSIONS

1. The method of Nelson and Samuels has been utilized in the study of the adrenal cortical response to surgery as measured by the circulating levels of free 17-hydroxycorticoids in serum. This method is chemically reproducible, and an index of the biologically active cortical steroids available to the patient at any given time.

2. The normal response to maximal ACTH stimulation and to surgical operations under general anesthesia has been defined. It is observed that the peak levels following surgery under these conditions are greater than

those produced by maximal adrenal stimulation with ACTH in the normal subject. Further, it has been found that a greater rise is produced by ACTH in the postoperative patient than in the normal or preoperative subject.

3. These findings are interpreted to indicate the presence of a delay in the intermediary metabolism or excretion of the free cortical steroids in the operated patient.

4. Further studies have been undertaken to explore the role of the specific effects of anesthesia and of tissue damage on the occurrence of this metabolic phenomenon.

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ADRENOCORTICAL RESPONSE TO SURGERY IN ELDERLY PATIENTS*

A. L. WATNE

Although many studies of the 17-ketosteroid and absolute eosinophil response to stress have been done, there is still some question as to the response of the adrenal cortex in geriatric patients as measured by these tests. This investigation was undertaken to compare the adrenocortical response to the stress of surgery in young adults with that response in geriatric patients.

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Studies have shown that with increasing age the daily 17-ketosteroid excretion in both elderly males and females decreased significantly. Fraser et al.[1] has shown that normal adult males in the third and fourth decades excrete an average of 13.8 mg. 17-ketosteroid per twenty-four hour period. Normal adult females excrete an average of 9.0 mg. 17-ketosteroid per twenty-four hour period. Hamilton and Hamilton[2] did studies of the daily ketosteroid excretion in normal males from 21 to 75 years of age. They found that the output of 17-ketosteroids during the seventh decade was less than half of that in the third decade Robinson,[3] on a similar study, found the 17-ketosteroid excretion in males to rise rapidly at the time of puberty and to reach its maximum in the fourth decade, followed by a gradual and progressive decline. Kenigsberg, Pearson, and McCavack[4] reported a significant decrease with increasing age in males, but not in females.

Forbes et al.[5] and Bennett and Moore[6] established the normal 17-ketosteroid response to stress to be an increase to well above normal within twenty-four to forty-eight hours after the stress. This is followed by a fall to a low point in four to five days, with a gradual return to normal levels on about the tenth day. Forbes found that chronically ill or severely undernourished patients had a remarkably low daily output, with no significant peak or fall following stress.

Thorn's work[7] on eosinophil response to adrenal cortex stimulation by ACTH and the correlation of this response as an index of the capacity of the adrenal cortex to excrete 11-oxysteroids is well known. The eosinopenia seen following stress is attributed to the increased output of 11-oxysteroid by the adrenal cortex, following surgery there may be a complete disappearance of circulating eosinophils during the first twenty-four to forty-eight hour period. Usually a sharp rise in circulating eosinophils, followed by the "backswing overshoot" as described by Moore,[8] occurs between the fourth and seventh postoperative days. Thus a finding of a normal or high eosinophil level during the first twenty-four to forty-eight hours after surgery would suggest adrenocortical insufficiency.

METHODS

The control series includes two males, aged 20 and 22, and three females, aged 35, 36, and 40. There are six elderly males with ages ranging from 61 to 76 years, and seven elderly females with ages ranging from 64 to 80 years. All the patients in both series sustained the stress of a major surgical procedure under general anesthesia. On each patient the twenty-four hour urinary excretion of 17-ketosteroids was determined for one to three days prior to surgery and for as long as sixteen days following surgery. Each twenty-four hour urine specimen was preserved with concentrated HCl, the collection being made at the bedside. Aliquots of each of these specimens were stored in the refrigerator until analysis could be carried out. The total neutral 17-ketosteroid excretion was determined by the acid hydrolysis and ether extraction method as developed by Dr. A. B. Kendrick, Department of Medicine, University of Illinois Research and Educational Hospital.[9] The final colorimetric determination was a modification of the technique of Holtorff and Koch.[10]

The absolute eosinophil count was also determined daily on each patient A daily fasting venous blood sample was drawn at 8 A.M. for this determination. Oxalate was used as the anti-coagulant. All the determinations were

made by the author. The staining of the eosinophils was done by the Henneman modification of the Randolph technique.[11] Counts were made on a Neubauer hemocytometer, and the total number of eosinophils per cubic millimeter of circulating blood were calculated according to Muehrcke.[12]

RESULTS

All the patients in the control groups survived a major operative procedure, and show an increased excretion of 17-ketosteroids and a marked drop in circulating eosinophils immediately following surgery. These findings are similar to those of other authors.[5,7]

The average twenty-four hour preoperative excretion of 17-ketosteroids for the control and elderly male groups is 15.52 mg. and 8.16 mg., respectively. The average preoperative absolute eosinophil count for the control

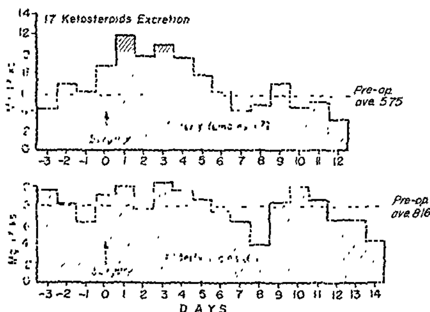


Fig. 1 The average daily 17-ketosteroid excretion for the elderly age groups. Both males and females show an increased excretion following surgery. The response in the elderly females is more marked.

males is 287 cells per cubic millimeter, as compared to 268 cells per cubic millimeter for the elderly males. The average daily preoperative excretion of 17-ketosteroids for the three control females is 5.72 mg compared to an average preoperative excretion for seven elderly females of 5.75 mg. per

perative abso-

, compared to

es.

tive and post-

operative periods for elderly males and females is shown in Figure 1. Both groups reveal a rise in excretion of 17-ketosteroids following the surgery, which lasts between one and five days. Following the initial rise there is a

level. The elderly females

s for the groups studied are

shown in Figure 2. There is a striking similarity between the response curve to stress of the elderly patients and their controls. The trend for a marked fall in circulating eosinophils appears in all groups, and is most marked twenty-four hours following surgery. There then develops an eosinophilia between the fourth and sixth postoperative days.

Of the six elderly males and seven elderly females studied, one male and one female died during the postoperative period. The daily 17-ketosteroid excretion and eosinophil count for these two patients is shown in Figure 3. Both patients had a very low preoperative absolute eosinophil count which fell to still lower levels in the immediate postoperative period. The male, N.M., who underwent a gastroenterostomy and cholecystogastrostomy for

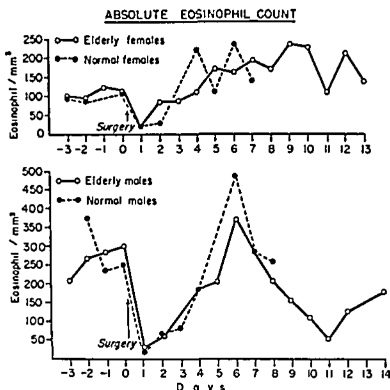


FIG. 2. The average daily absolute eosinophil counts for the elderly patients and their control groups. All the groups show a marked fall in the twenty-four hour period following surgery.

an obstructing duodenal carcinoma, showed an increase in 17-ketosteroid excretion on the third postoperative day. On the thirteenth postoperative day, a feeding jejunostomy was made. An eosinopenia again developed, but the 17-ketosteroids remained low. He expired on the sixteenth day following the initial surgery. No autopsy was performed. The elderly female, C.J., with carcinoma of the cecum, had an abnormally low preoperative absolute eosinophil count, which fell to still lower levels following surgery. The 17-ketosteroid excretion rose markedly on the first postoperative day, and remained elevated well above the average level for this age group, until her demise from cardiac failure on the fourth postoperative day. Her circulating eosinophils remained decreased throughout this period.

Three elderly patients, one male and two females, failed to show an in-

crease in 17-ketosteroid excretion following the surgery. All three showed a marked fall in circulating eosinophils The response of one of these patients, N.M., is shown in Figure 4.

DISCUSSION

The decreased daily 17-ketosteroid excretion found in the older age group is concurrent with the findings of other authors [2, 3] The average normal

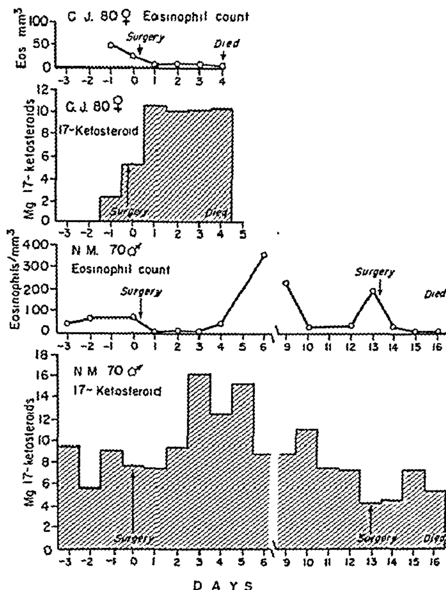


Fig 3. The daily 17-ketosteroid excretion and absolute eosinophil counts for the two elderly patients who died following surgery. The upper graph is of C J., an 80 year old female, who showed an increase in 17-ketosteroid excretion and a low eosinophil count until her death. The lower graph is of N.M., a 70 year old male, who showed a low eosinophil count and an increase in 17-ketosteroid excretion following the first surgery, however, at the time of his second operation the eosinophil count again decreased, but the 17-ketosteroid excretion remained at low levels until his death.

levels for the control males agree with levels given by others.[1] The reason for the slightly lower daily 17-ketosteroid excretion for the control females is unknown; however, this group is too small to be significant. The similarity in the daily average absolute eosinophil counts for the control and the elderly patients agrees with the findings of Dingwall, Heinzen and Pifer.[13]

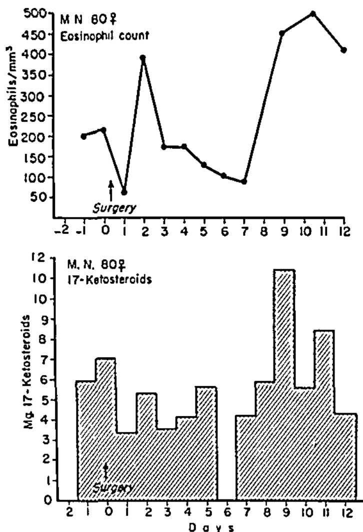


Fig. 4 The daily 17-ketosteroid excretion and absolute eosinophil count of M.N., an 80 year old female who underwent a common duct exploration with removal of a left hepatic duct stone This graph is typical for the three patients who showed no increase in 17-ketosteroid excretion following surgery, accompanied by a fall in the eosinophil count. She was weak and taking fluids poorly on the fourth postoperative day. Her appetite and strength improved and she was ambulatory on the eleventh postoperative day

Thus it appears that the average geriatric patient, 60 years or older, shows an adrenocortical response to stress sufficient to give a marked eosinopenia, similar to that seen in young adults.

The low preoperative levels of circulating eosinophils seen in the two patients who died might be attributed to the emotional stress of hospitalization. Both patients showed a response, as indicated by an increase in 17-

crease in 17-ketosteroid excretion following the surgery. All three showed a marked fall in circulating eosinophils. The response of one of these patients, N.M., is shown in Figure 4.

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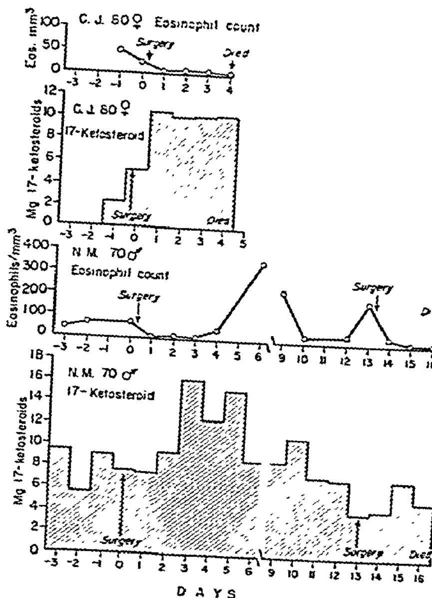


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FREEDOM FROM TETANY AFTER HOMOLOGOUS GLAND TRANSPLANTATION*

JULIAN A. STERLING AND RALPH GOLDSMITH

Transplantation of homologous thyroid gland was done in November, 1952, from an infant (21 days old) to a 29 year old female who had hypoparathyroid tetany of ten years' duration. The woman, since transplantation, has been free from symptoms of hypoparathyroidism. Coincidentally she has had relief from hypothyroidism. The patient is blood type AB, Rh positive.

[illegible]

spasm, parathormone was administered, oral calcium and vitamins were given. Within several weeks, tracheotomy was necessary. After attempts, over several months, to permit natural recovery, rehospitalization was required (medical school affiliate) in order to reposition the left vocal cord to permit decannulation.

For several years the patient was relieved of symptoms by the use of oral calcium, dihydrotachysterol and vitamins. Occasional intravenous calcium was required to control tetany, particularly during menses. However, with increase in weight, skin and hair [illegible], it was necessary to start therapy for [illegible]

[illegible] In 1949, transplantation of homologous parathyroid gland was attempted (at still a third institution), using a tissue culture-in-serum technique. This failed.

[illegible] Albert Einstein [illegible] of 10 per cent [illegible] This therapy was supplemented by the following daily medication: (1) dihydrotachysterol (AT-10), 2 to 4 cc; (2) viosterol, 1 to 3 cc, (3) calcium gluconate, 2 gm. 4 to 5 times; (4) thyroid extract, gr. ii, 2 to 3 times.

Positive Chvostek's and Trousseau's signs were almost constantly obtained.

* From the Albert Einstein Medical Center, Philadelphia, Pennsylvania.

ketosteroid excretion and further decrease in circulating eosinophils. The elderly female, C.J., maintained the low absolute eosinophil count and the elevated excretion of 17-ketosteroids until her death due to cardiac failure. The elderly male, N M, did not show an increase in 17-ketosteroids excretion following the second operation, but did show a fall in circulating eosinophils. The lack of response of the 17-ketosteroid excretion suggests that the adrenal glands may have been at the point of exhaustion. Cortisone therapy might have been beneficial.

The three patients who failed to show a response as indicated by 17-ketosteroid excretion, but who did show a fall in circulating eosinophils are similar in the fact that they all had a prolonged convalescence. Their postoperative course was marked by extreme weakness, with the need for supplemental intravenous fluids even after oral feedings were begun. It is possible that ACTH, given to stimulate the activity of the adrenal cortex, or cortisone to supplement the cortical hormones might have been beneficial. However, with the tests used here it is not possible to predict which patient would be benefited.

ndication for
. The eosino-
ity for these
patients However, the question remains whether hormone therapy would have helped the convalescence of the three patients who showed no response as measured by the 17-ketosteroid excretion, and the elderly male, who showed no increase in 17-ketosteroid excretion following the second operation.

SUMMARY

1. Studies of 17-ketosteroid excretion and absolute eosinophil counts were made on thirteen elderly patients undergoing major surgical procedures, with five young adults serving as the control group

2 The average daily excretion of 17-ketosteroids is lower for the elderly males than for young adult males The average daily excretion of 17-ketosteroids for the elderly females is approximately the same as that for the young adult females in this series.

3 The response to the stress of surgery of the elderly patients as measured by the twenty-four hour excretion of 17-ketosteroids is in general similar to that of the young adult patients

4. The absolute eosinophil response to stress for the elderly males and their control group, and for the elderly females and their control group follows a very similar pattern All the groups show a marked fall in circulating eosinophils following stress

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JULIAN A. STERLING AND RALPH GOLDSMITH

Transplantation of homologous thyroid gland was done in November, 1952, from an infant (21 days old) to a 29 year old female who had hypoparathyroid tetany of ten years' duration. The woman, since transplantation, has been free from symptoms of hypoparathyroidism. Coincidentally she has had relief from hypothyroidism. The patient is blood type AB, Rh positive.

Case Report. In April, 1941, this patient was treated at another institution for symptoms
...e. Immediately following this operation,
In addition, it was noted, bilateral recur-
...enous calcium was used to control tetanic spasm, parathormone was administered, oral calcium and vitamins were given. Within several weeks, tracheotomy was necessary. After attempts, over several months, to permit natural recovery, rehospitalization was required (medical school affiliate) in order to reposition the left vocal cord to permit decannulation.

For several years the patient was relieved of symptoms by the use of oral calcium, dihydrotachysterol and vitamins. Occasional intravenous calcium was required to control tetany, particularly during menses. However, with increase in weight, skin and hair changes, and other evidences of hypothyroidism, it was necessary to start therapy for this condition in 1944.

Medical management was increasingly difficult. In 1949, transplantation of homologous parathyroid gland was attempted (at still a third institution), using a tissue culture-in-serum technique. This failed.

In 1952 this patient was reporting to the emergency room of the Albert Einstein
30 to 40 cc. of 10 per cent
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Positive Chvostek's and Trousseau's signs were almost constantly obtained.

* From the Albert Einstein Medical Center, Philadelphia, Pennsylvania.

Transplantation was done November 21, 1952. Since then, the patient has taken no specific medication except for an occasional oral dose of calcium. Since the ninth postoperative day, intravenous calcium has been required on two occasions only. These occurred within four months of the transplantation and followed cystoscopic manipulation of intraureteral calculi. At least three urinary calculi have been recovered.

Genitourinary symptoms of pyelonephritis and cystitis have been treated by ammonium chloride and Terramycin based upon studies of bacterial flora sensitivities. Because of slight anemia, crude liver extract was given (intramuscularly) for about eight months after transplantation. At present, the patient takes liver and iron orally. She is also taking a high protein diet, including eggs and milk.

Severe dental caries has required total extractions with replacement by plates. The patient has received topical medication for recurrent nasopharyngitis and granular tracheitis. Politzerization has also been required.

Since fractures of the ninth and tenth dorsal vertebrae were incurred in 1948, the

Table 1 Laboratory Studies in Patient Who Received Homologous Thyroid Gland Transplantation (11-21-52)

DATE	AUG 1948	FEB 1950	NOV (28) 1952	DEC 1952	NOV. 1953	JULY 1954	OCT. 1954
Serum calcium (mg %)	5.7	7.8	15.6	9.7	11.8	8.5	9.0
Serum phosphorus (mg %)	6.4	4.5	3.4	3.8	3.9	2.8	4.5
Serum proteins (gm %)	8.9	6.0	5.0	7.2	7.9	6.0	8.5
Alkaline phosphatase (B units)		3.2			1.7		0.7
Serum cholesterol (mg %)		117			250	171	216
Plasma chlorides (mg %)					118		
Plasma sodium (mg %)					135		130
Plasma potassium (mg %)					3.9		4.9
Urine 17-ketosteroids (mg)					4.9		
Urine estrogen (mouse units)					30		
Urine gonadotrophin (mouse units)					32		
Remarks	****Tetany**********No tetany————						

patient has had low back pain. This is usually relieved by strapping and by a rigid supporting garment. Persistent low-back pain is the patient's only complaint at this time.

In July, 1954, 18 months after transplantation, appendectomy was required for acute gangrenous appendicitis. Operative and postoperative course has been completely uneventful.

She had been married (age 17) prior to the original thyroidectomy. She was divorced following onset of physical incapacity. At present, the menstrual cycle varies from 27 to 46 days, generally at a 31 day interval. Duration is five days; moderate cramps and frequent clots are present. Just before and during the early days of menstruation, the patient notes that the site of gland transplantation in the left groin swells slightly and is tender. She is able to do a full day's housework in caring for her home.

Prior to transplantation, her weight varied between 136 and 139 pounds. Following the procedure her weight was 133 pounds for about four months. Thereafter, she lost weight, so that at about 8 months after transplantation it was 107 pounds and during the past year and a half has been maintained between 103 and 105 pounds.

Prior to transplantation the patient's serum calcium was low (Table 1). Despite a level

attacks of tetany. 10 mg. per cent. er, tetany in this marked hypoproteinemia. When serum protein level is normal and the calcium is normal, there is no tetany in this patient. At present her calcium ranges from 8.5 to 9.0 mg. per cent, the is between 6.0 and 6.5 ky units. It is now 0.7

Two years prior to transplantation, while taking six grains of thyroid extract daily, her lantation it was bolic rates were 15 to plus 10.

Measurements of plasma sodium, potassium and chlorides are normal. Studies of hormone excretion were normal except for slight decrease in urinary estrogens.

Radioiodine tracer studies were done at 10 days and at three months after transplantation. These indicated 9 per cent and 6 per cent uptake, respectively, at the site of the transplanted gland. No other site of active thyroid tissue was observed.

TECHNIQUE

To accomplish the transplantation, several new concepts were applied. (1) Homologous gland was procured from an immediately deceased infant in order to utilize the most efficient activity of the "chief" cells in the parathyroid gland. (2) Gland nutrition and improved local drainage was provided by removing the parathyroid glands together with the intact thyroid gland and by using the major vascular pedicles of the thyroid gland for anastomoses with the host.

Under aseptic precautions, the infant's neck was hyperextended. Collar incision was made with oblique extensions along the sternocleidomastoid muscles. These and the ribbon muscles were cut to expose the carotid sheath and the thyroid gland. Carotid arteries were ligated distal to the origin of the superior thyroid arteries and as close to the clavicle as possible. Jugular veins were ligated superior to the middle (lateral) thyroid vein and low in the neck. Search was made for the thyroid ima and the inferior thyroid arteries; these, of tiny caliber, were ligated close to the thyroid gland.

The right side was dissected first. The middle thyroid vein and the jugular vein were freed from fascial and other connections. The common, external and internal carotid arteries were freed from fascial and vascular attachments. The left side was similarly prepared. The gland was freed posteriorly, leaving all areolar tissues attached to it.

The removed thyroid gland was about 2 cm. in diameter. Its estimated weight was 1.5 grams. The carotid arteries were approximately 5 mm. in diameter; the jugular veins were approximately 4 mm.

Incisions were made into the jugular and carotid vessels to insert polyethylene tubing. Residual blood was irrigated and a slow continuous drip of heparinized (1 mg. per cubic centimeter) Darrow's solution started into the arterial catheters. The gland was placed into this solution at room temperature and the catheters in the veins drained freely into the bath.

The left inguinal canal of the patient was opened under local anesthesia. Three vessels were exposed: probably the superficial external pudendal vein and the superficial external epigastric artery and vein. The external epigastric vein was cut to provide medial and lateral cut ends for use in vascular anastomoses. Serrefines were applied.

The inguinal canal was cleared of lymph nodes and areolar tissue in the region of the external ring to provide a bed for the transplanted gland. The gland was placed *with its anterior surface on the bed.* The end of the left carotid artery of the gland was joined to the side of the superficial external pudendal vein using five through-and-through fine silk sutures. Upon release of the vessel clamps, blood entered the transplanted gland. The lateral cut end of the epigastric vein was anastomosed to the left jugular vein by four silk sutures (Fig. 1). In each case, the polyethylene catheter was removed and the vessel cut to provide a non-traumatized lumen for anastomosis. Upon completion of these two anastomoses the gland changed from yellow to pink.

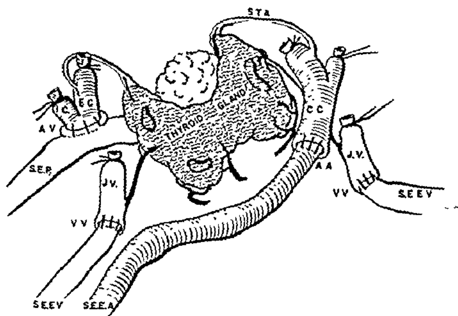


Fig. 1. Diagram of anastomoses between vascular pedicles of homologous gland and host vessels *A A*, End-to-end anastomosis between common carotid artery and superficial external epigastric artery *A V*, End-to-side anastomosis between common carotid artery and superficial external pudendal vein *C C*, Common carotid artery. *E C*, External carotid artery *I C*, Internal carotid artery *J V*, Jugular vein. *S.E E A*, Superficial external epigastric artery *S E E.V*, Superficial external epigastric vein. *S E P*, Superficial external pudendal vein. *S T A*, Superior thyroid artery. *V V.*, End-to-end anastomosis between internal jugular vein and superficial external epigastric vein.

The right jugular vein of the gland was then joined to the medial cut end of the superficial epigastric vein. The right carotid artery of the gland was anastomosed to the superficial external epigastric artery by end-to-end anastomosis. Only five sutures of fine silk could be placed. The carotid artery of the gland was larger than the host epigastric artery, mattress sutures were placed to occlude the excess (laterally) and a small piece of Gelfoam tied between long ends of adjacent sutures. Upon release of the serrefines, blood was seen to flow through the arterial anastomosis. The gland became reddish purple.

Operation for removal of the gland began one hour after the child's death. Transplantation was completed five and one-half hours later.

SUMMARY

A 29 year old woman had suffered from tetany due to surgical hypoparathyroidism for more than ten years. These symptoms were relieved following transplantation of homologous thyroid (including parathyroid) gland. Coincident relief of hypothyroid symptoms was noted. Observation in this case has extended to two years since the gland transplantation.

The method used in this case utilized young tissue. The gland was transplanted to the patient by means of anastomoses between the major vascular pedicles of the gland and the patient's vessels.

A LYMPHATIC FUNCTION TEST*

DAVID M. C. JU, ARTHUR BLAKEMORE, AND THOMAS W. STEVENSON

In blood there are three kinds of particles according to their size, namely, the electrolytes, molecules, and microscopic particles.

The electrolytic particles are small in size; they are freely permeable through the wall of the capillaries. There is always an equilibrium existing between the electrolytes in the blood and the tissue fluid. The concentration of these particles in both blood and tissue fluid is identical. The osmotic pressure exerted by these particles on both sides of the capillary wall is, therefore, equal. Generally speaking, when the electrolytic balance of the body is normal as in most cases of lymphedema, the electrolytes do not play an important role in the formation of lymphedema.

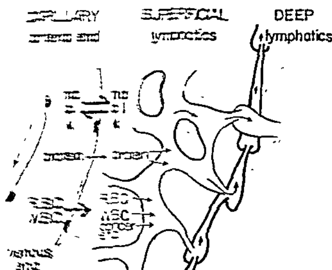
The molecular particles in blood, chiefly the protein particles, are large in size, and, generally speaking, the capillary wall is not permeable to these. However, there is evidence that even in normal conditions a small amount of protein particles, particularly albumin, does leak out through the holes of the capillary wall into the tissue space. Evidence shows that, because of the size of the albumin protein particles and because of the positive hydrostatic pressure in the capillaries, these protein particles in the tissue space are not reabsorbed by the blood stream. The lymphatic capillaries are known by their high permeability. The protein particles are freely absorbed through the lymphatics and from the lymphatic system they are transported back to the venous system through the lymphatic duct.

In addition to the osmotic pressure exerted by the electrolytes in the tissue fluid which are in equilibrium to those in the blood, the amount of osmotic particles in the tissue space depends on the one hand on the amount of leakage of protein from the vascular bed and on the other hand on its removal from the tissue space by the lymphatic circulation. The algebraic sum of these two factors will determine the amount of osmotic particles in tissue space and the ability of the tissue space to hold fluid.

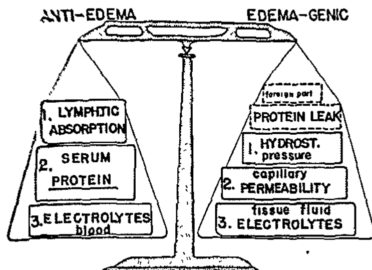
If the protein leakage is increased, as in different inflammatory conditions, allergic reaction, or anoxemia, the osmotic particles are increased in the tissue space. More fluid is held up and an edema results. However, if the lymphatic function is competent, in due time the excessive amount of protein in the tissue space will be drained away and the edema will accord-

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[illegible] On the other hand, when the lymphatic function is impaired, [illegible] permeability of the capillary wall is normal, because of the [illegible] small amount of protein into the tissue space, gradually [illegible] accumulate and a chronic lymphedema is the result.



[illegible] lymph: Electrolytes are freely permeable through the capillary wall [illegible] these particles in tissue fluid and in plasma is identical A small amount of protein, particularly albumin, under ordinary conditions leaks out from [illegible] the tissue space. These protein particles are absorbed only through [illegible] RBC and WBC leak into the tissue space only in abnormal conditions.



[illegible] 4. Since the osmotic pressure exerted by the electrolytes in the blood and tissue [illegible] equally identical in cases of lymphedema, the cause of disturbance of fluid balance [illegible] protein leakage and/or reduced lymphatic absorption In lymphedema there [illegible] mulation of protein particles in the tissue space and, therefore, the [illegible] pressure to hold fluid.

To better understand the mechanism of any case of chronic edema, we should either measure the actual amount of protein leakage or determine the rate of lymph absorption. Clinically it is impossible to determine the protein leakage. However, a lymphatic function test is possible. Since the

	Protein Outpouring	—	Lymphatic Drainage	=	FLUID in tissue space
	(when no Na retention) (serum protein normal)				
1. Normal	(+)	—	(++)	=	NO edema
2. Venous block	(++)	—	(++)	=	NO edema
	(+++)	—	(++)	=	EDEMA
3. Lymphatic block	(+)	—	(+)	=	NO edema
	(++)	—	(+)	=	EDEMA
	(+)	—	(0)	=	EDEMA
	(++)	—	(0)	=	

Fig. 3. Accumulation of protein particles in tissue fluid is the result of the algebraic sum of two factors protein leakage from the blood stream on the one hand, and lymphatic drainage on the other hand. Since the protein leakage under ordinary conditions is a slow and minimal process, even when the lymphatic system is totally obstructed, the accumulation of protein particles in the tissue space is a slow process However, once it is established it is permanent.

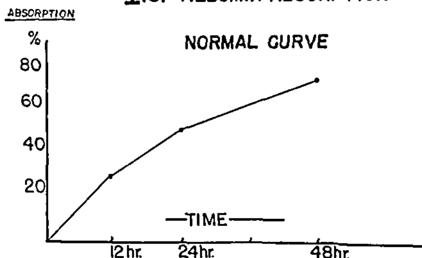


Fig. 4. Two cc of serum, 3 cc of saline, and 40 microcuries of I^{131}-albumin mixed and injected subcutaneously The absorption of the protein is followed by the study of the disappearance of radioactivity of I^{131} The natural decay was deducted according to the half-life curve of I^{131}. Control tests were done in twenty-five cases of apparently normal extremities The normal curve is established as above.

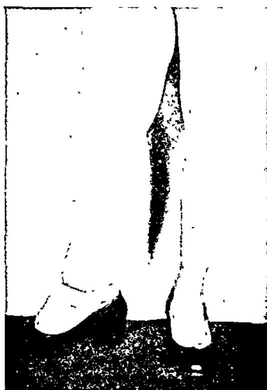


Fig 5. This woman patient had chronic lymphedema of her right lower extremity as a result of filariasis Studies of the cardiovascular system and the blood chemistry showed normal findings Lymphatic function test was abnormal.

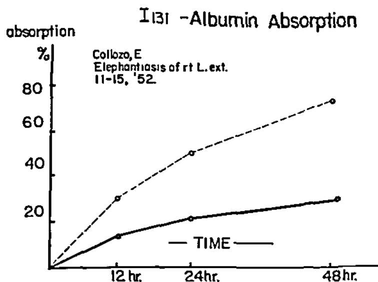


Fig 6. Lymphatic function test of the previous patient showed a flat absorption curve. Less than 30 per cent of the protein injected was absorbed in forty-eight hours. The lymphatic function is very much impaired.

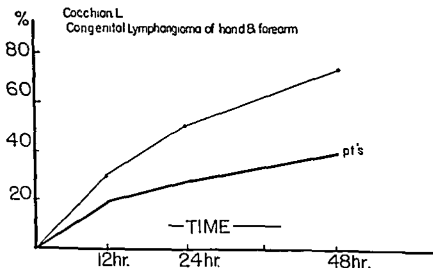
chief function of the lymphatic system is the removal of protein particles, if a known amount of the patient's own serum protein is injected into the tissue space and its absorption rate is carefully followed by some laboratory means, a lymphatic function test is thus feasible.

From animal experimentation it was definitely proved that radioactive iodinated albumin (I^{131}-albumin), which is a stable chemical compound, is absorbed largely through the lymphatic system and not through the venous



Fig. 7. A little girl born with a congenital lymphangioma of the right upper extremity. The diagnosis was established by biopsy and by pathologic study. Lymphatic function test showed a flat absorption curve.

I 131–ALBUMIN ABSORPTION



F

patient with congenital lym-
nction The lymphatic spaces
spaces of lymph. ... They are merely mechanical

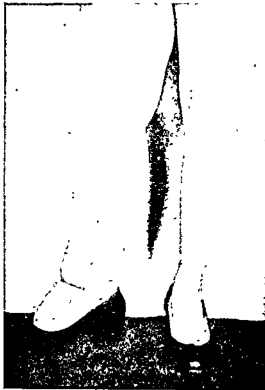


Fig 5 This woman patient had chronic lymphedema of her right lower extremity as a result of filariasis Studies of the cardiovascular system and the blood chemistry showed *normal findings Lymphatic function test was abnormal.*

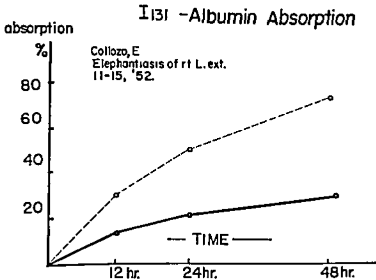


Fig. 6. Lymphatic function test of the previous patient showed a flat absorption curve. Less than 30 per cent of the protein injected was absorbed in forty-eight hours The lymphatic function is very much impaired.

been predicated primarily on the basis of cadaver studies, little is known about alteration of lymph flow in the presence of cancer, previous inflammatory disease, or when alternate routes are available. Operative procedures at present based on such rigid studies have become stereotyped and do not take in consideration any possible modification of lymph flow. To be able to determine the direction of lymphatic flow, therefore, would be of considerable value in planning an appropriate surgical attack for malignant disease.

Treatment of cancerous lymph nodes beyond the limits of surgical excision is in general unsatisfactory. External radiation therapy even with extremely high voltage has not given too much success. Since the radioactive colloids have been demonstrated to be phagocytized by the reticulo-endothelial cells of the body,[1-3] treatment of lymphatically disseminated cancer has been suggested by several investigators[2, 4, 5] in the treatment of inoperable carcinoma of the lung, cervix and prostate. Animal and human experiments with such radioactive material have disclosed the feasibility of irradiation damage, even to the production of complete necrosis in lymphatics and lymph nodes.[3, 4] In these instances, radiogold has been used and has been found located in the subcapsular and hilar regions of the lymph nodes as well as the reticulo-endothelial pathways which surround the follicles. However, little is known about the continued phagocytic function of a lymph node containing metastatic cancer and at what point such a node is so altered in its function that it no longer acts as a filter. While no deleterious effects on the metastatic neoplasm have been demonstrated, the few studies that have been reported are somewhat conflicting.[6] Obviously, more precise information is requisite before treatment of lymphatically disseminated cancer by radioactive colloids can be intelligently advised.

This study was therefore designed to explore further these concepts by attempting (1) to determine the prevailing routes of lymphatic drainage in human cancer as modified by the presence of lymph node metastases, and (2) to appraise the phagocytosis of radiocolloids by lymph nodes containing cancer.

METHODS

Radioactive gold was considered the most suitable colloid for these purposes. As received, it has a particle size of roughly the order of magnitude of 50 to 100 $m\mu$[7] and has been found to follow lymphatic pathways with phagocytosis by reticulo-endothelial cells. Dilution of the radiocolloid was carried out with 1 per cent procaine to provide a specific activity of from 0.1 to 5 millicuries per cubic centimeter. Initially, a dosage of from 2 to 5 millicuries was employed, but it has been found possible to procure satisfactory distribution and appraisal with as little as 0.1 millicurie. As supplied,* the colloidal solution is sterile, pyrogen free and stable on dilution with procaine. Tracer amounts of radiogold in a volume of 1 ml. were injected within 1 to 2 cm. of superficially located neoplasms such as those originating in the head, neck, or breast.

Surgical removal of the primary lesion, including the injection site and regional lymph nodes, was usually carried out within 20 to 24 hours following injection of the radioactive gold. In a few instances, operation was

* Abbott Laboratories.

system. Two cc. of patient's own serum, 3 cc. of saline, plus 40 microcuries of I^{131}-albumin are mixed and injected subcutaneously into the dorsum of the hand or of the foot; the amount of absorption of the injected material can be traced accurately by the radioactivity of I^{131}-albumin. After a series of measurement at different time intervals, the amount of protein absorption can be calculated and plotted on a curve. In normal cases, in twelve hours there is about 30 per cent absorption of the injected protein; in twenty-four hours, 50 per cent, and in forty-eight hours, 75 per cent or more. This is the normal lymphatic absorption curve.

This lymphatic function test was carried out in different patients with edema, some due to filariasis, others due to cancerous infiltration in the lymphatic system, others due to venous obstruction; a flat curve was invariably obtained in these cases. In patients with venous obstruction but no lymphatic impairment, the normal curve was usually obtained.

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THE USE OF RADIOGOLD IN STUDYING THE DYNAMICS OF LYMPHATIC DISSEMINATION OF CANCER*

COLIN G. THOMAS, JR.

The surgical treatment of cancer has been directed toward removal of the primary neoplasm en masse with its regional lymphatic drainage. When metastases have not occurred, or remain localized to accessible lymph nodes, such treatment seems to be effective Unfortunately not all neoplasms fall into this category. Frequently progressive growth of a metastasis results in obstruction of afferent pathways to a particular node, necessitating the participation of alternate collateral channels and the involvement of new and/or distant nodes. Since the "normal" routes of lymphatic drainage have

* From the Department of Surgery, University of North Carolina School of Medicine, Chapel Hill. The study was supported by a United States Public Health Service Grant-In-Aid

quantitative variation in radioactivity was considerable (Fig. 2) withou there being any well defined relationships to size of lymph nodes or the location. Involvement of secondary pathways such as the internal mammar lymph nodes or axillary nodes of the opposite side could not be demon strated in these individuals.

In a patient with only one axillary node metastasis, there was no demon ... patient with three axillary lym ymphatic flow to the area of t h extensive involvement of ax lary lymph nodes by cancer (10 of 16 nodes examined) had no depositi of radiogold in any of the axillary lymph nodes while the injection si remained high in residual radioactivity.

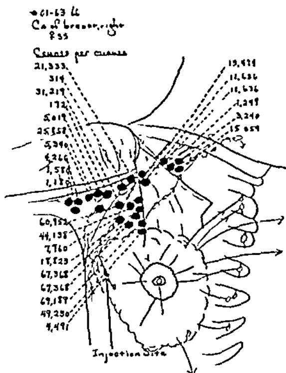


Fig. 2. Quantitative variation in the phagocytosis of radiogold by lymph nodes in co per minute per lymph node. (Same patient as Fig. 1.)

The time between injection and the operative procedure seemed to m little difference in the distribution of the radiocolloid. Furthermore, t seemed with one exception to be an immediate uptake by lymph nodes v rapid diffusion from the injection site. Consequently, in many instan lymph nodes had a count per minute as high as or higher than did ti from the injection site The rapid diffusion noted here, in contrast to observations of others, may be related to the deposit of radioactive g near rather than within the neoplasm.

From these data on patients with a breast cancer, the primary lymph flow appears to be in the direction of the axillary lymph nodes. (Note in none of these cases was the gold placed in the medial aspect of breast.) The data suggest that alteration in flow occurs only after ra extensive involvement by carcinoma.

deferred from 72 to 120 hours. Examination of the surgical specimen was performed within 24 hours, all grossly identifiable lymph nodes being removed by sharp dissection from the fresh specimen, their location plotted on an appropriate diagram and radioactivity assayed. Activity determinations were made with a gamma sensitive Geiger-Muller tube with a collimated beam. All tissues from the surgical specimen, including those from the region of injection site, were examined histologically for lymphoid elements, tumor, and relative amounts of each. Dark-field examination of the tissue sections disclosed the location of radiogold as highly refractile, spherical or cylindrical bodies When feasible, patients were surveyed pre- and postoperatively for location and intensity of the radiogold. Clinical

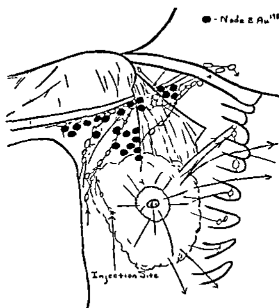


Fig 1 Location of lymph nodes ... with carcinoma of the outer quadrant of ... was injected in the outer quadrant of the ... and appraisal of surgical specimen were performed on the following day. Of 24 lymph nodes identified, all contained radiogold and none contained metastatic cancer.

determinations on patients were conducted with a directional Geiger-Muller tube or scintillation counter

OBSERVATIONS

Routes of Lymphatic Drainage. These could best be studied in those patients with carcinoma of the breast. Data on eight patients were suitable for analysis. Of these, five had no evidence of carcinomatous involvement of axillary lymph nodes Deposition of radiogold occurred throughout the axillary lymph nodes, namely the anterior, lateral, posterior, and apical groups of nodes (Fig. 1). There was little evidence of filtering of the colloidal particles by proximal nodes, although in one instance the subpectoral nodes disclosed a more uniformly high ... 2). In only one patient did all lymph ... gold. Of the 89 nodes examined in all ...

to demonstrate only a general localization of the radiogold in the region of the injection site and homolateral lymph nodes.

The Phagocytosis of Radiogold by Lymph Nodes Containing Cancer. Owing to the relatively high incidence of metastasis in those patients with cancer originating in the head and neck, this analysis concerns only these individuals. Of 109 lymph nodes examined, 47 contained appreciable deposition of radiogold. In 14 of these, the lymph node also contained carcinoma. Ten lymph nodes contained only carcinoma (Figs. 3 and 4).

Dark-field examination of the lymphatic tissue disclosed a very striking concentration of the gold particles by the reticulo-endothelial cells. In the

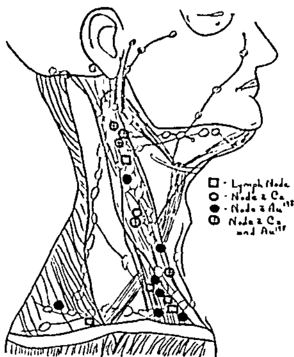


Fig. 4. Distribution of radiogold in a 67 year old male with carcinoma of the tonsil. Five millicuries of radiogold injected in region of the neoplasm on 1/14/54 Resection of neoplasm in conjunction with radical neck dissection performed on 1/18/54. Survey of patient on 1/29/54 disclosed moderate activity in region of tonsil and right supraclavicular region.

absence of any carcinoma, there was a fairly widespread distribution of the radiogold within the lymph node. Metastatic cancer can almost completely replace a lymph node with evidence of lymphatic occlusion, but if there is any remaining lymphoid tissue, it appears to be capable of retaining its filtering function and phagocytizing appreciable quantities of radiogold (Fig. 5). In several instances (Fig 4), only a rim of lymphatic tissue remained, but this disclosed intense activity. With the quantity of radiogold employed, as well as its brief period of action, there was little discernible effect on the structure of the lymph node and none on the contained metastatic neoplasm.

Examination of lymph nodes containing cancer but no radiogold disclosed a few with apparent complete replacement by neoplasm but most had generous amounts of lymphoid elements present. Some of these lymph nodes

In cancer of the head and neck, seven patients were available for study: carcinoma of the floor of the mouth—2, carcinoma of the parotid—1; carcinoma of the tonsil—1; and carcinoma of the hypopharynx—3. The results here were in many ways similar to those in carcinoma of the breast except that the disease in general was more advanced, with only one patient having no metastasis and one patient having only one metastasis. Again, the distribution of the radiogold was general, although concentrated primarily along the internal jugular nodes and somewhat less in the supraclavicular nodes of the posterior cervical triangle. Alteration in lymphatic flow could

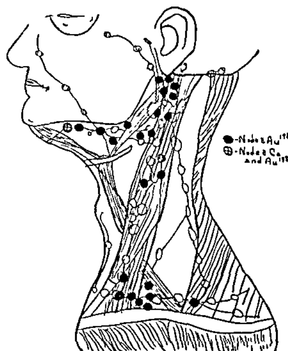


Fig 3. Location of lymph nodes containing radiogold in 41 year old male with carcinoma of floor of mouth Five-tenths millicurie of radiogold injected in region of carcinoma on 3/15/54 Excision and radical neck dissection performed on 3/18/54 Twenty-four lymph nodes were identified with one submaxillary node being partially replaced (½) by carcinoma All nodes contained radiogold with striking quantitative variation Tissue from injection site assayed at 8,533 counts per minute C p m in nodes varied from a low of 53 to a high of 55,700 The node containing metastatic carcinoma had a count of 12,800

not be demonstrated even though two patients required bilateral neck dissections because of metastases. In no instance was there any evidence of a "first line of defense" in which the radiogold was filtered so that more distant nodes were uninvolved This distribution, as in carcinoma of the breast, may be related to the particle size, since in the intravenous administration of radioactive colloids, the larger particles have been shown to be removed more rapidly than the smaller ones [7]

A survey of these patients was somewhat disappointing in our ability to localize accurately areas of radioactivity. The anatomy of the head and neck does not lend itself as well to such analysis as does the anterior chest wall, axilla, and supraclavicular areas It was possible by the means employed

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FLUORESCENCE OF HUMAN LYMPHATIC AND CANCER TISSUES FOLLOWING HIGH DOSES OF INTRAVENOUS HEMATOPORPHYRIN* †

D. S. RASMUSSEN-TAXDAL, GRANT E. WARD, AND FRANK H. J. FIGGE

It had been demonstrated that porphyrins have a tendency to accumulate in neoplastic, embryonic, and traumatized regenerating tissues of several species of animals.[1-3] It had also been shown that hematoporphyrin accumulated in lymph nodes and lymphatic tissues and tissues with a high mitotic index in general.[5] Attempts to utilize these findings in a practical way in 3 human subjects undergoing operations for head and neck cancer were unsuccessful.[5] One patient had received 30 mg. of hematoporphyrin, another received 60 mg. and a third received 120 mg. of hematoporphyrin intravenously. With these dosages neither the tumors nor the lymph nodes exhibited a sufficient degree of red fluorescence to be detectable.

In view of the spectacular results in animals, this result was not understood. A careful study of the history of the patients involved revealed that the head and neck regions had been irradiated with x-ray prior to the porphyrin injections. It was postulated that such radiation procedures might have suppressed mitotic activity in these tissues and thus destroyed their affinity for porphyrin. Hematoporphyrin, however, accumulated in all lymph nodes of animals after partial body irradiation. The dosages of hematoporphyrin used in the case of animals were, however, proportionately much higher than the 120 mg. used on the human subjects. It was, therefore, thought that it might be feasible to demonstrate lymph nodes and cancer tissues in human subjects if the dosage could be increased to 500 or 1000 mg. Even though relatively high doses of porphyrins have been employed in dogs, rabbits, mice, rats, monkeys, and guinea pigs, it seemed desirable to proceed with caution in elevating the dose in human subjects, because of the reports of the toxicity of hematoporphyrin which appear in literature.[4] The primary purpose of this investigation was to ascertain if the

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† This paper also appears in Cancer, 8 78–81, 1955.

were shown microscopically to contain material that looked like the gold particles, yet there was no significant radioactivity. Lymph nodes containing neither radiogold nor metastatic neoplasm disclosed no abnormality nor did these lymph nodes seem to have any particular pattern of distribution. As in the lymph nodes, the radiogold in the region of the injection site was also contained in large mononuclear phagocytes.

SUMMARY

Tracer amounts of radiogold injected near accessible neoplasms of the head, neck, and breast, diffuse rapidly from the injection site with phago-



Fig. 5 Photomicrograph of dark-field examination of carcinomatous node containing radiogold Tumor in center is encircled by highly refractile gold. × 25

cytosis occurring throughout the "regional" lymph nodes. Quantitative variation in the amount of radioactive gold present in the lymph nodes did not follow any definite pattern of distribution. Although complete replacement of a lymph node precluded the deposition of the gold colloids, the presence of only a small amount of lymphoid tissue renders a carcinomatous node capable of phagocytizing appreciable quantities of radiogold. Thus, radiogold is not only phagocytized by normal nodes, but may be deposited in those nodes containing cancer as long as there are any remaining lymphoid elements

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the lesion and its adjacent areas in the operative field. As the specimen was removed, it was more carefully examined and dissected under the spot beam in a completely darkened room. It was then possible to record pictorially the red fluorescence by employing a special photographic technique* as follows:

A. *Specimen Photography.* Specimens were photographed in a dark room using two near-ultraviolet spot lights as the exciting source. A 5 inch lens set at f 4.7 with a 7 inch bellows extension was employed. The system contained a 2A filter, and the film was Ektachrome daylight. Exposures varied from 1 to 2 minutes, depending upon the amount of fluorescence.

B. *Patient Photography.* This technique was developed to record those cases where the relatively long exposure time was impossible. A 5 inch lens set at f 4.7 with a 7 inch bellows extension was again employed. The 2A

Table 1. Summary of Cases Studied

CASE NO.	POSTOPERATIVE DIAGNOSIS	HEMATOPORPHYRIN DOSAGE	RED FLUORESCENCE
1	Squamous cell carcinoma of the tongue	300 mg.	+
2	Squamous cell carcinoma of the tongue	500 mg	+
3	Adenocarcinoma of the prostate	500 mg	−
4	Squamous cell carcinoma of the penis	500 mg.	+
5	Adenocarcinoma of the sigmoid colon with extension to the urinary bladder	500 mg	+
6	Ependymoma of cervical cord	500 mg	−
7	Fibrotic breast abscess	500 mg	−
8	Olfactory groove meningioma	500 mg.	+
9	Adenocarcinoma of the rectum	1000 mg.	++
10	Adenocarcinoma of the ascending colon	1000 mg.	++
11	Carcinoma of the breast	1000 mg.	++

filter was present as before. However, this time the exciting light was generated by a No. 50 flash bulb. A Corning filter No. 5970 covered the flash lamp. Exposure was made by open flash.

Successive increments of hematoporphyrin were used in order to cautiously observe tolerance. The first patient received 300 mg.; the next 7 patients received 500 mg., and the last 3 patients received 1000 mg. of hematoporphyrin. There were two reasons for increasing the dosages in these patients: (*a*) no toxicity was noted when the smaller doses were given; (*b*) the 300 mg. and 500 mg. dosages produced encouraging results, but it was felt that these could be enhanced with larger doses.

Case No. 4, diagnosed as a carcinoma of the penis, afforded us the following information. Due to a technical error, this patient received his 500 mg. of hematoporphyrin in three hours instead of six. At the conclusion of this procedure, a near-ultraviolet black light was directed at the lesion, at which time a bright red fluorescence was noted in the involved area. This indicated that there was a very prompt uptake of hematoporphyrin by a neoplastic tissue, and it further revealed that a fairly rapid administration was without untoward side effects.

* ... tion extended by C. F. Reather and ... ne Johns Hopkins School of Medicine ... s investigation. They deserve special credit for suggesting the special flash photographic technique for use in operating rooms and on living subjects.

dosage of hematoporphyrin could be increased in human subjects to a level sufficiently high to be practical for demonstration of lymphatic and neoplastic tissues. It will be shown that it is indeed possible to give as high as 1 gm. of hematoporphyrin by the intravenous drip method over a 24 hour period. It was also possible to demonstrate that when sufficiently high doses of hematoporphyrin are administered, the lymphatic and cancer tissues of human subjects fluoresce a brilliant red as compared with normal tissues of other types. Thus, it has been possible to utilize this method to demonstrate and delimit lymphatic and cancer tissue in human subjects.

MATERIALS, METHODS AND RESULTS

Eleven human patients, whose ages ranged from 46 to 76, were studied in this series. The criteria for selecting a patient for this study were as follows: (1) non-sensitivity to hematoporphyrin as demonstrated by an intradermal skin test, and (2) suspicion or definite knowledge of carcinoma.

The intravenous route of administration was employed in all cases. Forearm veins were chosen because of the relatively long administration period. This interfered least with the patients' feeding and excretory problems. A No 20 needle was introduced into the vein, and the drip rate was set between 15 and 20 drops per minute. On the first few patients to receive the hematoporphyrin, the blood pressure and pulse were noted every fifteen minutes during the administration. Both pressure and pulse remained stable, and it was further noted that there was no temperature aberration during or after the infusion.

The chemical used in all cases was hematoporphyrin hydrochloride (recrystallized). A unit for injection was prepared by dissolving the desired amount of hematoporphyrin in 600 cc of M/6 sodium lactate, to which had been added 1 gm of sodium bicarbonate. The hematoporphyrin was found to be readily soluble in this alkaline medium (pH 7.6). It should be noted that the solution used in these experiments contained no phenol and thus differs from commercially available hematoporphyrin solutions The first unit contained 300 mg. of hematoporphyrin, whereas all other units contained 500 mg of the drug. The resulting solution was then doubly filtered (through filter paper) and bottled. The unit was then sterilized in a standard autoclave for fifteen minutes at 250°C.

A standard blood administration set was used to introduce the solution intravenously. The time of administration varied from three to ten hours with an average of six hours Because it was sometimes impossible to know of the planned surgery more than twenty-four hours beforehand, some cases received their hematoporphyrin on the afternoon or evening prior to surgery. Others, because of cancellation of cases for various reasons, received their hematoporphyrin as much as seventy-two hours prior to surgery.

In order to demonstrate fluorescence, a near-ultraviolet spot light was employed. The light was generated by a G.E reflector spot quartz mercury-arc light, medical unit. A bright red fluorescence was indicative of a relatively high concentration of hematoporphyrin. Patients were examined under this spot light prior to receiving hematoporphyrin in order to detect any naturally occurring red fluorescent areas Some typical porphyrin red fluorescence was most commonly noted at the nasolabial folds (sebaceous secretions) and on the posterior portion of the tongue.

At the time of operation, the near-ultraviolet spot beam was directed at

activity. Concurrent with this symptomatology, the patient developed anorexia and weight loss. Because of the persistence of these symptoms, the patient presented himself to the accident room, where x-rays and later a barium enema revealed a right colon filling defect. The patient had no bowel irregularities, melena, or indigestion. His weight loss in 5 months was 16 pounds.

On 3/1/54, the patient was given 500 mg. of hematoporphyrin solution intravenously by slow drip over an 8 hour period. On 3/2/54, this procedure was repeated with a 5 hour administration period. On 3/3/54, the scheduled day of surgery, the case was cancelled. On 3/4/54, the patient was taken to the general operating room. As the peritoneum was incised and the right colon exposed, the room was made dark and an ultraviolet spot light was

noted that it was possible to fluo-

the lesion was dull red at this time.

l was longitudinally incised, it was

possible to view the fungating cauliflower lesion in toto. When the ultraviolet light beam was directed at this lesion, it was noted to be intensely red fluorescent. The surrounding tissues did not fluoresce red. Again no toxic effects of this high dosage were noted.

Case No. 9. H.D., J.H.H. 662139, a 76 year old colored male. Six months prior to admission to the hospital, the patient had an episode of loose stools tinged with blood. The patient also noted that he had been losing some weight. The diarrhea cleared with conservative treatment, and the patient described his general health as good. Two weeks prior to his admission, he was seen by his physician who noted an abdominal mass and subsequently referred him to the hospital. Here it was further noted that a rectal mass was present. This mass was biopsied and proved to be adenocarcinoma.

On 2/24/54, the patient received 500 mg. of hematoporphyrin intravenously in the usual manner over a 6 hour period. On the morning of 2/25/54, this procedure was repeated with an additional 500 mg. of hematoporphyrin. On 2/25/54, the patient was taken to the general operating room where an abdominal incision was made for exploratory purposes. After the peritoneum was incised, a loop of bowel was held up with its attached mesentery. A large nodule was present in the mesentery, and the ultraviolet light was directed at this area. The nodule showed bright red fluorescence and was later confirmed as being metastatic carcinoma. It was interesting to note in this case that the lymph vessels leading to this lesion also stood out as bright red strands. The surrounding tissues did not fluoresce. No toxicity was noted in this patient.

SUMMARY AND CONCLUSIONS

A series of 11 patients have been given injections of hematoporphyrin intravenously in dosages varying from 300 to 1000 mg. These dosages are far in excess of those previously thought to be toxic, but there has been no evidence of toxicity in any case. The first case to be studied received 300 mg; the next seven cases received 500 mg., and the last three cases received 1000 mg. of hematoporphyrin. In those cases receiving less than 1000 mg. of hematoporphyrin, the ability to demonstrate red fluorescence in lymphatic and cancer tissues was good but was not considered optimal. In those cases in which 1000 mg. of hematoporphyrin was given, the demonstration of red fluorescence in lymphatic and cancer tissues was considered excellent.

Cases 5, 8, and 1 received 300 to 500 mg. and were all successful in that the lesion fluoresced red under near-ultraviolet light, but lymphatic systems were poorly visualized in cases 5 and 1.

The carcinoma of the prostate (case 3) did not visibly fluoresce red either at the time of operation or when the specimen was taken to the dark room. Grossly the tumor did not appear malignant. The pathologic report, however, revealed a "small adenocarcinoma with invasion of the capsule and hyperplasia." Further studies will be necessary to determine whether any or all types of prostatic neoplasms have any tendency to accumulate hematoporphyrin.

The ependymoma of the cervical cord (case 6) did not become red fluorescent within 24 hours after the administration of 500 mg. of hematoporphyrin. Why this tumor failed to fluoresce is not understood.

The only other tumor which did not fluoresce red was that of Case 7. This had been diagnosed preoperatively as a carcinoma of the breast, and, therefore, it was expected that it would be red fluorescent. However, no red fluorescence was observed, and the lesion was finally diagnosed as a fibrotic breast abscess.

Cases Receiving 1000 mg. of Hematoporphyrin. *Case No. 11.* M.L., J.H.H. 665882, 51 year old colored female. Seven months prior to admission, this patient noted a firm non-tender mass in the left breast about the size of a walnut. Because the mass continued to grow in size, the patient consulted her physician, who referred her to the hospital. She had also noted a lump in her axillary region. The patient was admitted to the hospital with the presumptive diagnosis of carcinoma of the left breast.

On 3/8/54, the patient was tested intradermally with hematoporphyrin solution and found to be non-sensitive. Following this procedure, the patient was given 500 mg. of hematoporphyrin dissolved in 600 cc. of M/6 sodium lactate containing 1 gm. of sodium bicarbonate. This solution was administered by slow intravenous drip over an 8 hour period. The following morning the above procedure was repeated again using 500 mg. of hematoporphyrin. On the afternoon of the same day, the patient was taken to the general operating room. After routine preparation of the patient, the operating room was made dark, and the near-ultraviolet spot light was directed on the exposed left breast. It was possible at this time to see beneath the

turned back on and a small incision was made in the lower outer quad-
for the purpose of obtaining a confirmatory frozen section. The tissue removed was noted to contain hematoporphyrin as evidenced by red fluorescence under ultraviolet light. A radical mastectomy was then performed. Two nodes fluoresced in the axilla, and we were able to see lymph vessels clearly showing bright red fluorescence under ultraviolet light. Following the block dissection of the lesion, the specimen was then cut across, and the porphyrin content of the lesion, as evidenced by red fluorescence, was noted to be high. Definite red fluorescence was observed throughout the entire tumor. Color photographs of the fluorescing phenomena were taken. No toxicity from the increased dosage of hematoporphyrin was noted.

Case No. 10. W.S., J.H.H. 582122, a 49 year old colored male. Five months prior to admission, this patient noted a gradual onset of soreness in the right side of his abdomen. This complaint was unrelated to meals or

Methods of Procedure and Materials.

A synthetic diet comparable to the U.S.P. vitamin A test diet was developed. All essential nutrients for mice except vitamin A were included. The composition of the experimental diet was as follows:

Cane sugar	58%
Vitamin free casein	16%
Crisco	8.7%
[illegible]	3.5%
[illegible]	10%
[illegible]	2.1%
Dried yeast (Fleischmann 2019)	1.4%
Viosterol (vitamin D)	2 cc. per batch

Dry ingredients were weighed and mixed in a McClellan Batch Mixer for 1/2 to 1 hour. A small aliquot of the mixed dry feed was ground in a mortar and pestle with 1.5 grams of crystalline methylcholanthrene. This pulverized aliquot was thoroughly blended into the total quantity of dry ingredients using the batch mixer. Melted Crisco was allowed to cool and the viosterol added, the fat [illegible]

[illegible]

majority of animals had xerophthalmia, loose starry hair, scaly tail skin, and weight loss. [illegible] *'s on nutritious diet. It was necessary to* [illegible] deficiency diet and prolong life so that [illegible]

Results of Vitamin A Experiments. Squamous cell carcinomas of the forestomach appeared in the C_3H and A strain mice, but not in the DB strain. No adenocarcinomas of the stomach were found. The incidence of squamous cell lesions in the various groups was as follows: group A, 47 per cent; group AD, 45.6 per cent; group HAD, 9 per cent; and group ADC, 0 per cent. It is interesting to note that squamous cell cancer (induced by methylcholanthrene) appeared earlier in the mice on a vitamin A deficient regimen than in mice on a normal fully nutritious diet. The earliest squamous cell lesion appearing in group AD (carcinogen plus vitamin A deficient diet) at 62 days, and in group A (carcinogen plus nutritious diet) at 205 days. However, a high supplement of vitamin A in the diet did not significantly delay the appearance of these lesions, for in group HAD the earliest squamous cell cancer was found at 100 days. Of the mice in this group on the experimental regimen for 100 days or more, only 9 per cent developed squamous cell cancer.

In one case, direct visualization of the cancer tissue was possible through the skin and in another through the bowel wall.

It now appears to be possible to utilize the red fluorescence of hematoporphyrin and its tendency to concentrate in tumors to assist the surgeon to visualize and delineate neoplastic tissue during operations. The detection of small or obscure lymph nodes may also be facilitated by these methods.

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THE USE OF DIETARY DEFICIENCIES TO INFLUENCE THE ACTION OF METHYLCHOLANTHRENE UPON THE STOMACH OF MICE*

CLAUDE R. HITCHCOCK

The virtual absence of glandular gastric cancer in lower animals remains a stimulating enigma. Only an occasional glandular carcinoma can be induced in the prepyloric stomach of mice or rats with direct implantation of a powerful carcinogen (methylcholanthrene) into the mucosa.[10] Monkeys are resistant to the effects of such carcinogens in every tissue of their body.[8] During the course of experiments to attempt potentiation of carcinogen action upon the glandular gastric mucosa of mice, the use of dietary deficiency states appeared plausible.

VITAMIN A DEFICIENCY AS ABETTING FACTOR

Vitamin A is considered a catalyst of oxidation reduction reactions, and severe deprivation for prolonged periods of time produces marked tissue changes. In 1913 Osborne and Mendell first described xerophthalmia in rats due to vitamin A deficiency, and in 1925 Wohlbach and Howe demonstrated metaplasia of mucosa to squamous epithelium when rats were depleted of vitamin A.[14] Pappenheimer and Larimore, in 1924, reported raised plaques of hyperkeratosis and ulcerations in the forestomachs of rats maintained on vitamin A deficient diets.[7]

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vitamin A supplement with methylcholanthrene resulted in hyperplasia and hyperkeratosis in 11 per cent of the mice in group HAD.

Papillomas appeared in 20.1 per cent of the mice in group A, and 9.6 per cent of the mice in group AD. Mice in control groups ADC and HAD failed to develop papillomas. No significant sex differences were noted in these experiments.

Discussion of Vitamin A Deficient Experiment. It was anticipated that carcinogen and the vitamin A deficient diet would induce an appreciable number of benign changes in these mice, and this was substantiated. Also, the absence of papillomas in the mice supplemented with vitamin A and receiving carcinogen is compatible with previous reports of a protective action of this vitamin in this regard. However, not only did excess vitamin A fail to prevent induction of squamous cell carcinomas of the forestomach, but the lesions appeared at relatively early age (100 to 121 days on experimental regimen). The vitamin A deficiency state had a significant effect, for squamous cell cancers of the proventriculus appeared much earlier after initiation of the experimental diet than they did when all essentials of the diet were present.

Forestomachs of the DB strain of mice were resistant to the induction of squamous cell cancers under all experimental conditions. This strain appears to have an unusually resistant squamous epithelium, and an interesting tendency of the glandular epithelium to hyperplasia. Microscopic examination of the glandular stomachs of the other strains of mice did not reveal significant histologic changes.

VITAMIN B DEFICIENT DIET AS ABETTING FACTOR

Various constituents of the vitamin B complex either inhibit or abet the action of certain carcinogens in some animal tissues. Thus, biotin, inositol, pyridoxine, and para-aminobenzoic acid have a procarcinogenic effect for butter yellow in the induction of cancers in the liver of the rat. Riboflavin and avidin are anticarcinogenic for this carcinogen. A deficiency of thiamine causes atony and relaxation of the intestinal musculature as well as suppression of gastric and pancreatic secretions. Deficiency of nicotinic acid (pellagra) produces severe gastro-intestinal changes in many animals including rats, dogs, and man.

Methods of Procedure and Materials.

A synthetic diet was designed to be deficient in vitamins of the B complex, while providing other dietary essentials.

Cane sugar	60%
Vitamin free casein	18%
Crisco	8%
Salt mix	4%
Cellu flour	8 6%
Yeast (St. D –360)	1.6%
Cod liver oil	9 cc.

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5 DB, group BDC:
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The addition of the vitamin A deficiency state to the effects of methylcholanthrene produced hyperplasia and keratosis of the forestomach in 42 per cent of the mice as compared with 35 per cent of the mice exposed to carcinogen in normal feed. When vitamin A deficient diet was fed alone, 1.8 per cent of the mice showed hyperplasia and hyperkeratosis. The high



a C_3H mouse containing a large, fungating, ys after feeding on vitamin A deficient diet

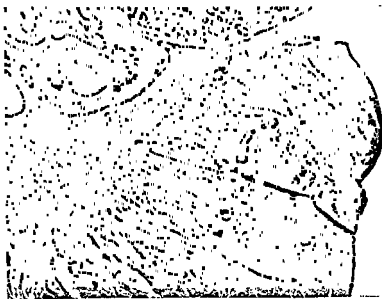


Fig 2 Photomicrograph of cancer in Figure 1 showing masses of malignant squamous cells growing beyond serosa of the proventriculus. Circa 40 ×.

ing the casein level to 18 or 20 per cent in order for normal growth to be maintained.

In 1936 Sharpless demonstrated papillomas, hyperplasia, and mucosal ulcerations in the forestomachs of rats fed a low casein diet. He found a 12 per cent casein diet, or the addition of 0.2 per cent cystine to the low casein diet, to be effective in preventing these lesions.[3]

Intimately associated with the effects of protein and specific amino acid deficiency is the effect of a below normal caloric intake. In 1943 Visscher, Ball et al. noted a marked reduction of spontaneous breast cancers in C_3H virgin female mice when the carbohydrates and fat in the diet were reduced sufficiently to produce a one-third reduction in daily calories.[11] Proteins, vitamins, and salts were maintained at normal levels in their experiments. The drop from 67 per cent tumors in the control mice to 0 per cent tumors in the calorie-restricted mice was striking. This result may well be due partly to fat restriction, since very low fat intake is now known to significantly postpone the appearance of both spontaneous and induced cancers.

Materials and Methods of Low Protein Experiment.

Mice of the C_3H, A, and DB strains were used. The control group A was the same as in the previous experiments. Group LP (low protein diet plus methylcholanthrene) consisted of 54 mice (29 C_3H, 16 A, 9 DB) The second control group, LPC (low protein diet alone), had 51 mice (5 C_3H, 8 A, and 38 DB). The synthetic diet was made to contain 6 per cent casein.

Cane sugar	55%
Vitamin free casein	6%
Salt mix (U.S P XII)	4%
Cellu flour	15%
Crisco	12%
Yeast (St. D.–360)	4%
Yeast (2019)	4%
Cod liver oil	6 cc.

Diet ingredients were mixed and prepared in the same manner as the vitamin deficient diets The concentration of methylcholanthrene, as in the other experiments, was 0.250 mg. per gram of feed. While these animals maintained their weight better than mice on the vitamin deficient diets, it was necessary to interrupt the feeding schedule with short periods on normal fox chow. Eleven such interruptions were made with an average of 10 days on normal diet each time. The mortality during the early days of the experiment was considerably less than in the vitamin deficiency experiments.

Results of Low Protein Experiment. The low protein and carcinogen diet induced squamous cell cancers of the forestomach, but the low protein diet alone did not. No adenocarcinomas of the stomach resulted from this experiment. Mice in group A ate the carcinogen fox chow diet for an average of 217 days, contrasted to an average of 163 days for mice in group LP. In group A 47 per cent of the mice on diet over 100 days had squamous cell cancers in the forestomach and in group LP 30 per cent of the mice in the same category had squamous cell cancers.

It is important to note, however, that the earliest lesion appeared in the LP group at 59 days after start of the experiment, while the earliest lesion in group A appeared at 205 days. Hyperplasia and hyperkeratosis of the forestomach mucosa was present in two-thirds of the C_3H strain mice in group LP. Papillomas also were common in this strain on the low protein diet with carcinogen. No papillomas or hyperplasia developed in the mice in control group LPC. These mice ate the experimental diet for an average

dence of nutritional deficiency. Periodic breaks in the feeding regimen were necessary every 20 to 30 days, and 12 such interruptions were made.

Results of Vitamin B Deficient Experiment. Squamous cell carcinomas of the forestomach appeared in mice on the carcinogen and vitamin B deficient diet, but not in the animals on vitamin B deficient diet alone. The incidence of squamous cell cancers in group A was 47 per cent of the mice on experimental diet over 100 days, in group BD 21.4 per cent of the mice on experimental diet over 100 days had squamous cell carcinomas. Here also the squamous cell cancers appeared in a shorter time in animals on the vitamin B deficiency diet (146 days for group BD) than in animals on carcinogen and a nutritious diet (205 days for group A).

The average number of days on the experimental diet of the mice that developed tumors was twice as great for group A mice as for group BD mice This indicates a likely potentiation of carcinogen action by the deficiency state. The difference between the incidence of squamous cell carcinomas of the forestomach in group A and group BD mice (group A, 46 3 per cent; group BD, 12 per cent) probably is owing to the much higher mortality of the mice on the deficiency regimen early in the experiment Also significant is the longer life span of animals on the nutritious diet (group A mice had cancers appearing as late as 507 days after initiation of the experiment) as compared with a markedly shorter life span for group BD mice The animals in group BDC also tended to die at an earlier age.

Papillomas of the forestomach as well as hyperplasia and hyperkeratosis appeared with almost identical frequency in group A and group BD mice. However, mice in group BDC (vitamin B deficient diet alone) did not develop papillomas or hyperplasia and keratosis of the ruminant pouch mucosa In addition, no dilute brown strain mouse developed cancer in the stomach of either the squamous or glandular type. No sex differences were noted in the groups of mice that died of squamous cell gastric cancer. The experiment failed to produce a glandular gastric cancer in any of the strains of mice used.

Discussion of Vitamin B Deficient Experiment. While no glandular cancers resulted, and the incidence of squamous cell cancers was lower in group BD mice than in the control group A mice, the vitamin B complex deficient diet most likely was responsible for the short induction time of squamous cell cancers in group BD It seems clear that the deficient diet in this experiment potentiated the action of methylcholanthrene upon the squamous cell mucosa of the forestomach, since no significant findings were present in the stomachs of mice eating the deficient diet without carcinogen. Mice in group BDC averaged a longer period of time on the carcinogen free deficient diet than did mice in group BD, and still no papillomas or keratoses were noted in the BDC animals

LOW PROTEIN DIET AS ABETTING FACTOR FOR METHYLCHOLANTHRENE

All tissues of the body depend in great part on the quality and quantity of protein in the diet for maintenance of cellular health If all essential protein constituents are fed to mice and rats in a diet offered ad libitum, the casein concentration can be lowered to 9 per cent and the animals will continue to grow normally. Restriction of the daily caloric intake necessitates increas-

not increase the over-all yield of squamous cell cancers of the proventriculus nor did they predispose the glandular prepyloric mucosa to adenocarcinoma.

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AN ANTI-TUMOR FACTOR IN THE BLOOD OF TUMOR IMMUNE RATS*

ROBERT SCHREK AND FREDERICK W. PRESTON

Homologous grafts of tumor tissue in animals, just as homologous grafts of normal tissue in humans, may grow at first and then regress. The animals are then highly resistant or immune to subsequent implants.

The nature of the immunity to transplantable tumors, or to grafts of normal tissue, is not understood. In general there are two schools of thought. One claims that the immunity is humoral and is caused by antibodies or cytocidal agents in the serum. The other school considers that the immunity

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of 174 days compared to 163 days for group LP (low protein diet and carcinogen). It seems clear that under the conditions of this experiment the low protein diet alone did not cause significant changes in either the squamous or glandular stomach of the mice. No sex differences were apparent in any group All stomachs exposed to carcinogen and low protein diet were sectioned for histologic study, and no demonstrable differences in the glandular mucosa could be found.

Discussion of Low Protein Experiment. These mice remained in surprisingly good condition considering the severity of the protein deficiency. This does not correlate with the findings of White et al. who noted marked and progressive reduction in weight when mice were fed a 5 per cent casein diet. It hardly seems possible that 1 per cent of casein in over-all concentration could account for such a difference. More likely the answer lies in variations in concentration of the remaining constituents of the diet.

Considering the appearance of the earliest squamous cancer in experi- [illegible] the time of appearance of the earliest lesion [illegible] iet appears to speed up the action of the car- [illegible] However, the deficiency state did not increase [illegible] ous cancers. The low protein diet did appear to increase the number of cases of multiple primary cancers in the same stomach.

It was surprising to note the absence of any pathologic changes in the stomachs of mice on the low protein diet alone. The small intestine as well as the colon was examined in every animal and was normal in appearance. Perhaps specificity of species is responsible for the difference between the response of the mouse and rat in this regard. It is possible, of course, that another strain of mouse, not tested in this experiment, might show squamous mucosal lesions similar to those reported for the rat

SUMMARY

Mice of the C_3H, A, and DB strains were subjected to experimental diets plus methylcholanthrene in the following manner. vitamin A deficient diet plus methylcholanthrene, vitamin B complex deficient diet plus methylcholanthrene, and low protein diet plus methylcholanthrene. Control groups consisted of mice on methylcholanthrene and nutritious diet, and mice on each deficiency diet without carcinogen.

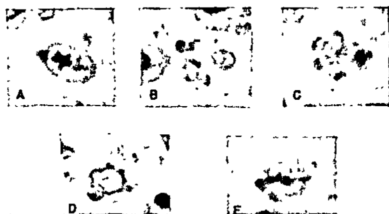
The action of methylcholanthrene on the glandular, or prepyloric, stomach of mice was not potentiated by diets deficient in vitamin A or vitamin B complex, or low in protein. No adenocarcinomas were produced. In the control groups, none of the deficiency states noticeably altered the histologic appearance of the glandular mucosa.

Squamous cell carcinomas of the forestomach were produced in all mice ingesting methylcholanthrene except the dilute brown (DB) strain. In control group A (full diet plus carcinogen) there were 47 per cent squamous cell carcinomas, vitamin A deficient diet plus carcinogen, 45.6 per cent; vitamin B complex deficient diet plus carcinogen, 21.4 per cent; low protein diet plus carcinogen, 30 per cent squamous cell cancers of the proventriculus. Each deficiency state caused squamous cell carcinomas to appear at a significantly earlier age than when nutritious diet was used with carcinogen (64 days after start of feeding to 205 days for appearance of first lesion). These deficiency states did speed up the action of the carcinogen, but did

added to the mixtures, and the unstained cells were counted in a hemocytometer. The cells resistant to staining were considered to be viable.

The results of one experiment are presented in Table 1. In this experiment, the sera from 5 immune and 3 normal rats were tested. The cell suspensions in normal sera were estimated to have an average of 2470 viable cells per cubic millimeter before incubation and 2170 after 5 hours of incubation. The difference in the average counts is not statistically significant. Apparently all or nearly all the cells in the control sera survived the incubation.

In 2 of the 5 immune sera, the viable cell counts even before incubation were low, 900 and 1100. These two immune sera were evidently rapidly cytotoxic. After 5 hours of incubation the viable cell counts were low in



chromatin masses. (194 minutes incubation.) *D*, The lobules have fused, resulting in a icubation.) *E*, The in structure. (211

4 of the 5 immune sera, 20 to 300, as compared to 1900 to 2500 cells in the normal. The fifth serum was not toxic. Thus in this experiment, 4 of 5 immune sera were cytocidal to tumor cells. In all, the sera from 23 immune rats were tested and cytotoxicity was demonstrated in fifteen (65 per cent).

It may be concluded that the sera of most, but not all, immune rats contained substances which were toxic in vitro to the cells of Bagg's lymphosarcoma according to the method of unstained cell counts.

TIME-LAPSE CINEMICROGRAPHY

The apparatus consisted of an inverted metallurgic microscope, enclosed in an incubator and equipped with phase optics, a 16 mm. movie camera,

B

nor

per cubic millimeter.

is cellular and is dependent on the reactions of certain cells, such as lymphocytes or plasma cells.

In a recent review of the subject, Prehn and Main[1] state that it is much simpler to discover circulating cytotoxins following heterologous transplantation of tissue than following homologous transplants. Although a few investigators have demonstrated cytotoxins in completely homologous systems, most investigators in this field have reported failure.

The problem of homologous cytotoxins to transplantable tumors has been re-explored in this laboratory with newly developed methods—namely the method of unstained cell counts,[2] phase microscopy and time-lapse cinemicrography.

THE METHOD OF UNSTAINED CELL COUNTS

The tumor used was Bagg's rat lymphosarcoma, reticulum cell type, which was originally obtained from Dr. Anna Goldfeder and which is de-

Table 1. The Effect of 5 Immune Rat Sera and 5 Hours of Incubation at 37°C. on the Number of Viable Cells in Suspensions from Bagg's Lymphosarcoma

SERUM		NO. OF VIABLE CELLS PER CU. MM. BEFORE INCUBATION	AFTER INCUBATION
Normal	1	1900	1900 (77%)*
	2	3000	2100 (85%)
	3	2500	2500 (101%)
Average		2470	2170 (88%)
Immune	1	2300	100 (4%)
	2	900	20 (1%)
	3	2600	300 (12%)
	4	1100	40 (2%)
	5	2200	1900 (77%)

* Percentages are based on the average count (2470) of cells in normal serum before incubation.

scribed by her.[3] The tumor was maintained in white Sprague-Dawley rats. [illegible] the tumor the rats were resistant to reimplantation. Soon after the tumors regressed the rats were bled from the heart under ether anesthesia and the serum was collected.

One method of testing the toxicity of the serum was the use of unstained cell counts, which has been described previously.[2] A suspension of tumor cells in Ringer's solution having a concentration of approximately 30,000 viable cells per cubic millimeter was prepared. Mixtures were made consisting of 1 part suspension, 5 parts fresh normal or fresh immune rat serum, 4 parts Ringer's solution and 1 part distilled water. The mixtures were incubated in a water bath for 5 hours at 37°C. with sufficient shaking to prevent settling of the cells. Eosin, 1 to 500, in Tyrode's solution was then

peripheral zone and a dark center (Fig. 2A). These cells did not have any pseudopodia or posterior tails and none showed ameboid motion. Each cell gradually developed a large, round, conspicuous, vesicular nucleus with a well defined nuclear membrane and a large, clear, structureless, vacuolar central area (Fig. 2C). A few dark chromatin masses were apparently adherent to the nuclear membrane. The vesicular nucleus persisted for a short period of time and then suddenly collapsed as if it had ruptured (Fig. 2D and E). It took 1 to 3 minutes for the nuclear membrane to collapse. The cytoplasm then contained the dark masses, previously observed in the nucleus (Fig. 2F). Gradually the dead cells underwent postmortem changes and their cytoplasm became more transparent (Fig. 2G). Ultimately the cells became small, with fine and coarse granules and with poorly-defined borders (Fig. 2H).

With most immune sera, all the tumor cells appeared small, shrunken, and dead within 5 hours. In contrast, the cells in normal serum were large and appeared healthy, with active ameboid motion at the end of this time interval. These findings indicate that the immune serum contained substances which were toxic to the tumor cells and which caused a distinctive type of death.

In other experiments, immune serum which was known to be cytocidal to tumor cells was heated at 56°C. for 30 minutes. The heated immune serum did not cause the morphologic changes described above, and did not kill the tumor cells but produced agglutination or clumping of viable tumor cells. When the clumping involved only two tumor cells, they were seen to adhere to each other by their tails. Both cells frequently maintained their motility and tended to move in opposite directions. This microscopic tug of war usually ended with one cell moving off in one direction and dragging the other one behind it. Large clumps involving many viable tumor cells were also formed and were usually in an active state of flux owing to the activity of the viable cells. Evidently, the immune serum heated at 56°C. for 30 minutes lost its cytocidal properties but demonstrated a capacity to agglutinate tumor cells.

PHASE MICROSCOPY

Time-lapse cinemicrography was useful for demonstrating the activity of immune serum, but was not convenient for testing a large number of sera or for studying the properties of cytotoxins. With the experience gained with cinemicrography, we were able to recognize the cytotoxic action of immune serum by direct observation with phase microscopy.

Tumor cell suspensions in immune serum were prepared in the same manner and in the same chamber as for cinemicrography. The preparations were examined with a phase microscope, usually after 4, 8 and 20 hours of incubation. Photomicrographs were made as a matter of record. Living and dead cells usually were readily differentiated. The viable cells were large, round or ameboid in shape with large nuclei and sparse cytoplasm. In immune serum, the dead cells were frequently small, dark, with many coarse granules and no distinct nuclei. Some dead cells had a group of eccentrically placed masses with varying amounts of light or dark cytoplasm. After incubation in normal serum, some cells were slightly irregular,

One drop (approximately 0 01 cc.) of this suspension was placed on a thin glass slide. Two drops of the immune or normal serum were added, and then one drop of Ringer's solution diluted with an equal volume of distilled water. The fluid was then covered with a cover slip. A metal ring 0.8 mm. in thickness was used to separate the cover slip and the slide. The preparation was sealed with paraffin and incubated on the microscope at 37°C. Pictures were taken at the rate of 1 frame per 6 seconds The magnification was 76× on the film and 800× on the prints in Figures 1 and 2

In normal serum, the tumor cells appeared large, with large nuclei and sparse cytoplasm. Many tumor cells developed small anterior pseudopodia and small posterior tails (Fig 1*A*) They maintained this polarity and moved in straight lines. Viable tumor cells which were not in ameboid motion usually had a slight vibratory, rhythmic movement. In normal serum,

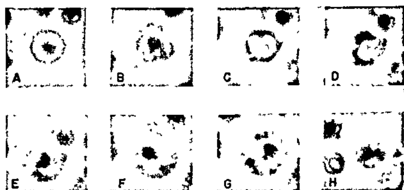


Fig 2 Death of a tumor cell (Bagg's lymphosarcoma) in immune rat serum showing successive degenerative changes Prints made from a cinemicrographic film *A*, The first stage of degeneration is a spherical cell with a bright peripheral zone (49 minutes incubation) *B*, The cell is irregular in shape (55 minutes incubation) *C*, The nucleus appears vesicular (88 5 minutes incubation) *D*, The nuclear wall begins to collapse (88 6 minutes incubation) *E*, The nuclear wall has completely collapsed, liberating the chromatin granules into the cytoplasm (88 7 minutes incubation) *F* and *G*, The round cell has a few chromatin granules and transparent cytoplasm (94 and 108 minutes incubation) *H*, The final stage is a shrunken cell with dark cytoplasm (172 minutes incubation)

nearly all the tumor cells survived 5 hours and many of the cells survived 24 hours.

The death of tumor cells in normal serum was usually associated with certain morphologic changes. An apparently normal cell would suddenly develop approximately 6 to 12 lobules (Fig. 1*B* and *C*). These lobulations were associated with rapid and extensive changes in the shape of the cell. Under favorable conditions, each lobule was seen to have a clear central intranuclear vacuole (Fig. 1*B*) This process continued for about one-half hour and then the cell would become quiescent and was obviously dead. The dead cell was round or slightly irregular in shape, and, with the phase objective, appeared bright, refractile and structureless (Fig. 1*D*).

When incubated with immune sera, tumor cells frequently showed early morphologic changes. Immediately after the preparation of tumor cells suspended in immune sera, many cells became spherical, each with a bright

2. Vycital, R. O., Schrek, R., and Clarke, T. H.: Unstained cell counts as a method of evaluating cancerocidal agents. J. Lab. & Clin. Med., 42:320–331, 1953.
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SERIAL TRANSPLANTATION OF HUMAN NEOPLASMS IN CORTISONE-TREATED HAMSTERS*

W. BRADFORD PATTERSON

In recent years, new methods for the heterologous transplantation of tumors have been developed which promise to be practical for widespread use. For the past eighteen months we have been transplanting human neoplasms into the cheek pouch of hamsters, a technique first described by Lutz et al.[1] Treatment of the animals with cortisone, as suggested by Toolan,[2] effectively blocks the inflammatory response of the hamster to foreign tissue, and markedly diminishes host resistance. The cheek pouch technique is simple and can be carried out by technicians after very little training. Sterile fragments of tumor are cut to fit into the end of No. 16 trocars and inserted under the mucous membrane of the everted pouch while the animal is anesthetized. The transplants can later be visualized and studied at any time without sacrificing the animal.

During the year ending in July, 1954, we transplanted fifty-five malignant tumors, freshly obtained from the operating room.† These transplanted tumors fall into three groups (Table 1). The first group includes those

Table 1. Unsuccessful and Partially Successful Transplants

	TYPE	SITE	NUMBER
Group I–Failures	Carcinoma	Breast	5
	Lymphoma	Nodes	3
	Epidermoid ca	Mouth	3
	Adenocarcinoma	G I. Tract	3
	Miscellaneous		9
	Total		23
Group II–Temporary survival	Fibrosarcoma	Trunk	3
	Epidermoid ca.	Lung, kidney, vulva	5
	Carcinoma	Breast	3
	Adenocarcinoma	Exocrine and endocrine glands	6
	Miscellaneous		9
	Total		26

* From the Department of Pathology, New England Deaconess Hospital and Department of Surgery, Peter Bent Brigham Hospital, Harvard Medical School, Boston, Massachusetts. This work was supported by funds from the American Cancer Society and the United States Atomic Energy Commission Contract AT(30-1)-901 at the New England Deaconess Hospital.

† At the New England Deaconess, the New England Baptist, and the Peter Bent Brigham Hospitals.

brilliant and structureless and resembled the cells which had recently died by lobulation as observed in cinemicrographic studies (Fig. 2C).

By these procedures, tests were made on sera obtained from 65 rats with regressed tumors. Forty-three of the sera (66 per cent) were toxic to the cells and killed 50 to 100 per cent of the cells in 4 hours, while normal sera allowed survival of 90 to 100 per cent of the cells in this time interval. This finding of 66 per cent positive sera as determined by the method of phase microscopy agrees well with the finding of 65 per cent positive sera by the method of unstained cell counts. Ten sera were tested by both methods, and identical results of toxicity were obtained

Titration of one active immune serum showed an appreciable cytotoxic effect when the final dilution was 1 in 32. This does not seem a high titer of activity With higher concentrations of sera, cytocidal activity could be readily recognized after 2 hours of incubation. With diluted sera, the most favorable time of observation was 8 hours

Direct observation confirmed the cinemicrographic findings that heating of immune sera to 56°C. for 30 minutes inhibited the cytocidal activity and caused clumping of tumor cells The heated immune serum regained its cytocidal properties upon the addition of an equal volume of fresh normal rat serum. The titer of the mixture of normal and heated immune serum was approximately the same as the titer of fresh immune serum. The observed inactivation of immune serum by heat and the quantitative reactivation with fresh serum are in accord with work on other antibodies, such as immune hemolysins. The findings suggest that immune homologous serum contains a cytotoxin or antibody which, together with complement, causes morphologic changes and death of the cells of Bagg's lymphosarcoma.

The capacity of various types of cells to absorb the cytotoxic factor was tested by mixing the sera with various cellular suspensions. After incubation for 1 hour at 17°C, the mixtures were centrifuged and the supernatant was tested for cytotoxicity. The cells tested included viable cells of Bagg's lymphosarcoma, the same tumor cells killed by heat, Walker rat tumor cells and lymphocytes prepared from normal rat spleen and thymus. Only the viable cells of Bagg's tumor removed the cytotoxic agent from the immune serum. Thus the cytotoxic agent seemed highly specific

SUMMARY

Rats with an acquired immunity to transplants of Bagg's lymphosarcoma were prepared by inoculation of the tumor into the rat and selecting animals in which the tumor grew and later regressed The sera of these rats were shown to be toxic to Bagg's lymphosarcoma cells.

The toxic action of this serum was studied by three in vitro methods. the unstained cell count method, direct observation with phase microscopy, and time-lapse cinemicrography Characteristic morphologic changes developed in the nuclei of tumor cells incubated with immune sera.

The cytotoxic factor in immune sera required complement for its cytocidal action. It was absorbed by viable Bagg's tumor cells but not by dead Bagg's tumor cells, cells of another tumor or by normal rat lymphocytes.

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During the year ending in July, 1954, we transplanted fifty-five malignant tumors, freshly obtained from the operating room.† These transplanted tumors fall into three groups (Table 1). The first group includes those

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† At the New England Deaconess, the New England Baptist, and the Peter Bent Brigham Hospitals.

tumors which were quickly absorbed by the hamster. Infection, rapid autolysis of tumor tissue while in transit, and various immunologic barriers may account for these failures.

Group II transplants survived for periods of twelve days to three months. This is the "bridesmaid" group, tumors which were unable or unwilling to form a permanent alliance with the hamster. These tumors did not appear to be actively rejected, but seemed to die gradually of starvation.

Table 2. Group III: Serially Transplantable Tumors

NAME	TYPE	PRIMARY SITE	DATE OBTAINED
Deac-1	Muco-epidermoid carcinoma	Parotid	Sept. 1953
Deac-2	Epidermoid carcinoma	Penis	Feb. 1954
Deac-3	Embryonal carcinoma	Testis	Feb. 1954
Deac-4	Epidermoid carcinoma	Mouth	Mar. 1954
Deac-5	Undifferentiated carcinoma	Prostate	June 1954
Deac-6	Carcinoma simplex	Endometrium	May 1954

Six tumors fell into Group III (Table 2). All of these show active steady growth and are serially transplantable. We are at present maintaining stock in these tumors at the New England Deaconess Hospital and a number are being utilized in other laboratories for experimental purposes. This group of lesions all came from patients in whom the cancer had metastasized or recurred The growth characteristics of our first serially transplantable tumor, Deac-1, are representative of the group. This muco-epidermoid car-

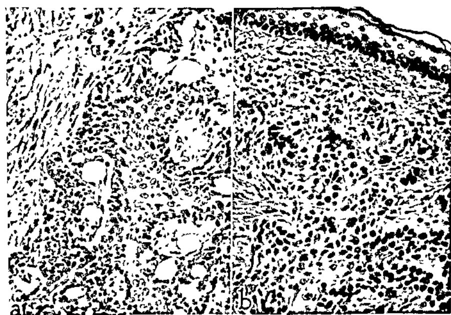


Fig 1 *a*, Deac-1: epidermoid carcinoma of parotid—original tumor. ×250 *b*, Deac-1. sixth generation transplant in hamster. ×250.

cinoma (Fig. 1) has been carried through more than twenty generations of hamsters in the past year. The histology of the tumor differs from that of the original specimen only in that no mucin is produced and occasional areas show the pattern of an adeno-acanthoma. Transplants of this tumor are successful in 75 to 100 per cent of animals and increase in size as much as a hundredfold during two to four weeks (Fig. 2*a*).

Efforts in our laboratory have been directed primarily toward enlarging this group of tumors. Our success thus far has been due in large measure to close rapport with the departments of surgery and pathology, which enabled us to obtain carefully chosen tissue specimens and have them transplanted within thirty to sixty minutes. Although speed may not always be



pouches *b*, Deac-1 transplant
Deac-3 embryonal carcinoma.
. ×200.

essential, the selection of viable tumor by its gross characteristics or by frozen section is crucial.

We have carried out a few pilot experiments with the Deac-1 tumor. Utilizing the unique anatomy of the cheek pouch, one can selectively expose growing masses of human tumor to x-radiation, while the host is completely shielded. Preliminary experiments suggest that the sensitivity of these tumors may not be significantly altered by transplantation to the hamster. For example, transplants receiving as much as 4500 r in divided doses* have shown nests of highly-differentiated tumor cells still apparently viable After establishing a base-line response for each tumor, we plan to investigate ways in which this response might be enhanced by treatment of the host.

on cortisone has been
with cortisone shows
t resistance (Fig. 2*b*).

* 140 kv., 5 ma., 15 5 cm. distance, HVL= 2.4 Al

A stalemate exists, with host reactive tissue effectively holding the healthy tumor in check. All of our tumors have continued to require cortisone even after repeated transplantations. We feel that the persistence of this cortisone dependency signifies that the transplanted tumor is still alien human tissue. If cortisone is stopped during the early growth phase, a host reaction develops which first limits and then destroys the tumor.

A critical question in the clinical application of the cheek pouch technique is whether these transplants react to various stimuli in the same manner as the tumor reacts in the patient. No experimental data have yet been reported which bear on this question. However, a striking phenomenon is frequently observed in sections of our embryonal carcinoma which suggests that this tumor is reasonably content in its substitute host and might, therefore, be expected to react as it does in the patient. Tumor cells in these transplants are often clearly oriented in orderly fashion around the small blood vessels of the host supporting stroma (Fig. 2c). This has been called the "pigs at the trough," and would indicate that the cells either relish or need what is being supplied by the host In view of this obvious compatibility, it seems reasonable to predict that the cheek pouch blood vessels offer a new and direct pathway by which to explore and to influence the metabolism of human tumors. Further studies aimed toward early clinical application are therefore being pursued with these transplantable human tumors.

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EFFECT OF ESTROGEN AND TESTOSTERONE ON RATE OF NUCLEIC ACID SYNTHESIS IN MOUSE MAMMARY CANCER*

LOUIS-PHILIPPE ALLEN, ALOIS VASICKA, AND SOMERS H. STURGIS

Steroid hormones have been noted to exert a powerful but occasionally unpredictable influence on the clinical course of certain cancers of the reproductive system.
breast appears to be
substantial number of
spontaneously developing mammary cancer in the females offer disadvantages as well as advantages in the study of this action. These tumors can be readily grafted into males of the same strain, but in both sexes may mature so rapidly towards a fatal termination that there is little opportunity to record gross effects of injected steroids It seems desirable to utilize

* From the Surgical Service of the Peter Bent Brigham Hospital, Boston, Massachusetts
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some indication of intracellular metabolism closely allied to tissue synthesis in working with these tumors. Since the nucleic acids DRNA and RNA are essential components involved in cellular proliferation, we have chosen to study the effect of estrogens and androgens on these compounds extracted from the mouse neoplasms in acute experiments. We have utilized P^{32} as a tracer to record the specific activity of phosphorus in the total nucleic acids fraction after steroid injections. The rate of incorporation of the isotope is considered a rough index of nucleic acid synthesis, and thus of growth rate of these carcinomas.

MATERIAL AND METHODS

A strain of mature C_3H mice bearing spontaneous mammary cancers in the females or grafted tumors in the males was used.* Castration was performed after tumors were grossly palpable to eliminate gonadal hormones. Ten days later, except as noted below, injections were given as follows:

Group I, controls, received 0.2 ml. sesame oil only for 3 days before sacrifice.

Group II received 2 gamma of estradiol dipropionate in sesame oil† on days 10, 11 and 12 except for modifications noted in the tables of results, and were sacrificed on day 13.

Group III received 2 gamma of testosterone propionate in oil† on days 10, 11 and 12 except as later noted, and were sacrificed on day 13.

From 3 to 10 mice were used in each control and experimental group. The tumors from the surviving animals in each group were pooled, after rejecting grossly necrotic or hemorrhagic areas.

In the males, it was found that the transplanted tumors often grew at such a rate that it was necessary to modify the above regime by giving the steroid injections on days 5, 6 and 7 and sacrificing on day 8 after castration.

[illegible] tissue for from [illegible] a Krebs-Ringer-[illegible] of P^{32} as phosphate.‡ These flasks were shaken at 37°C. in an atmosphere of air for 2 or 3 hours. The reaction was stopped by the addition of 7 per cent trichloracetic acid in the cold.

The flask contents were then washed with 4 volumes of cold 7 per cent TCA and homogenized in 10 to 20 volumes of the same solution. The phospholipids were removed by washing with 3:1 ethanol-ether, and the residue was extracted twice with 5 per cent TCA at 90°C. to yield the total nucleic acid fraction.

Phosphorus analyses were performed on aliquots of this fraction after the method of Fiske and Subbarow[1] following digestion with hot sulfuric acid and hydrogen peroxide. Aliqots of the nucleic acid fraction were counted for radioactivity in an end-window Geiger counter with appropriate corrections for absorption, background and coincidence. The specific activity (S.A.) of the nucleic acid phosphorus is expressed as counts per minute

* Animals were obtained from the Jackson Memorial Laboratory, Bar Harbor, Maine.

† Estradiol dipropionate and testosterone propionate were generously supplied by the Ciba Pharmaceutical Products, Inc, Summit, New Jersey.

‡ We are indebted to Dr. A K Solomon, of the Harvard Biophysical Laboratory, Harvard Medical School, for his aid and assistance in counting the isotope.

Table 1. Effect of Estrogen on Specific Activity of Nucleic Acid P in Mouse Mammary Cancer in Castrated Females

	EXPERIMENTS*								
	A[1]	B[1]	C[1]	D[1]	E[1]	F[1]	G[2]	H[3]	I[4]
Mice Controls	5	3	5	4	5	6	6	6	5
Experimental	4	3	5	3	5	7	7	3	6
Flasks Controls	4	3	5	5	10	6	6	6	6
Experimental	4	4	6	5	10	6	6	4	6
S A † of controls	316	197	142	79	395	822	822	822	547
S A † of experimental	388	232	183	109	463	985	909	1183	789
Percentage difference	+23%	+18%	+28%	+37%	+17%	+19%	+10%	+13%	+44%
Significance, *t* value	>4	1.8	>4	>4	2.5	1.9	0.9	3.7	3.4

*Dosage of estradiol dipropionate in sesame oil 1, two gamma daily for 3 days before sacrifice, 2, five gamma daily for 3 days before sacrifice, 3, ten gamma daily for 3 days before sacrifice, 4, ten gamma daily for 2 days before sacrifice

†S A. is relative specific activity, computed as the ratio of counts per minute per milligram nucleic acid P to the counts per minute per millimeter of media

per milligram phosphorus, related to the activity of the incubating medium. The level of S.A. in one experiment cannot be compared directly, therefore, with that obtained in another experiment with a different medium activity.

With each experiment a group of control flasks containing aliquots of pooled tumors that had not been treated was processed.

RESULTS

Females. In Table 1 the effect of estrogen is recorded in nine different experiments using castrated females. In every instance the injection of

Table 2. Effect of Testosterone on Specific Activity of Nucleic Acid P in Mouse Mammary Cancer in Castrated Females

	EXPERIMENTS*				
	A[1]	B[1]	C[1]	D[1]	[illegible]
Mice. Controls	5	5	4	3	10
Experimental	4	4	4	3	10
Flasks. Controls	4	5	5	3	10
Experimental	4	4	5	3	12
S. A. of controls	316	142	79	287	214
S. A. of experimental	309	136	79	176	169
Percentage difference	−2%	−4%	0	−58%	−21%
Significance, t value				2.6	3 0

* Dosage of testosterone propionate in sesame oil: 1, two gamma daily for 3 days before sacrifice; 2, three gamma daily for 2 days before sacrifice

estrogen before sacrifice was associated with an increased rate of incorporation of P^{32} into the tumor nucleic acids. In five of the nine experiments this increase was highly significant, yielding a t value of more than 3.

The effect of testosterone on similarly castrated females is recorded in Table 2. In none of the five experiments was there found an increased

Table 3. Effect of Estrogen on Specific Activity of Nucleic Acid P in Mouse Mammary Cancer in Castrated Males

	EXPERIMENTS*		
	A	B	C
Mice: Controls	5	4	10
Experimental	5	4	5
Flasks: Controls	4	4	8
Experimental	5	4	8
S A. of controls	349	347	150
S. A. of experimental	493	362	117
Percentage difference	+41%	+4%	−22%
Significance, t value	2 7	2 5	2.4

* Dosage of estradiol dipropionate in sesame oil, two gamma daily for 3 days before sacrifice.

specific activity of nucleic acid phosphorus in the tumors after androgen injection. Indeed, a statistically significant depression of this rate is seen in the last two experiments.

Males. When estrogen was given to castrated males bearing tumor transplants in three experiments, the results were inconclusive (Table 3). In experiment C the tumors grew so rapidly that only 5 out of 8 experimental

animals survived and the cancer tissue in those that did survive was grossly deteriorated with marked necrosis and infection at time of sacrifice.

Testosterone was given to castrated males in five experiments (Table 4). In three of the five, a highly significant increase in P^{32} incorporation over that of the controls was found. A depression was seen in experiment D, however, and again this was associated with an unusually rapid growth of

Table 4. Effect of Testosterone on Specific Activity of Nucleic Acid P in Mouse Mammary Cancer in Castrated Males

	EXPERIMENTS*				
	A[1]	B[1]	C[1]	D[1]	E[2]
Mice: Controls	5	4	4	8	10
Experimental	5	4	2	5	10
Flasks Controls	4	4	4	8	8
Experimental	5	4	3	8	8
S. A of controls	349	347	220	150	107
S A of experimental	531	1271	279	92	197
Percentage difference	+52%	+266%	+11%	−48%	+83%
Significance, t value	>4	>4	0 9	2 3	>4

* Dosage of testosterone propionate in sesame oil· 1, two gamma daily for 3 days before sacrifice, 2, three gamma daily for 2 days before sacrifice

the tumors, resulting in major breakdown of the tissue at time of sacrifice of those that survived

DISCUSSION

At the start of this investigation, certain observations were made on the effect of steroids using intact female mice It was found that some of these animals had conceived before they were shipped to our laboratory and were in different stages of gestation at time of sacrifice For this reason all preparations here reported were standardized in both sexes by castration and then hormone injections after an arbitrarily chosen and generally constant interval of 10 days. Yet, there were other variations in our material that undoubtedly contributed to the results In the first experiments on females we used spontaneous tumors in "retired breeders." These older animals occasionally succumbed to our experimental procedure Later, we found it more satisfactory to obtain young but mature vigorous virginal females, as well as males, none of which grossly bore any neoplasms.

With each shipment were included tumor-bearing "donors"; castration and transplantation were done simultaneously with invariably good "takes." In these young animals, however, the tumors at times outgrew their blood supply, and the resulting necrosis and infection were not infrequently fatal before conclusion of the 13 day post-castration span of our experiment. This was true particularly of the animals in experiment C, Table 3, and experiment D, Table 4, where, in each case, only 5 of the original 8 mice in these groups survived

Every effort was made to eliminate all grossly unsatisfactory tissue in surviving tumors from each tumor pool. Clearly the rate of incorporation of any element into an organic compound is basically a function of the viability of the tissue concerned A decrease in rate of P^{32} incorporation into the nucleic acids of these cancers should not then be interpreted as

necessarily due to steroid-induced inhibition of their growth. On the contrary, it could be that the given steroid caused such a marked acceleration of growth that by the elected time of sacrifice and assay the tumor had already reached a terminal phase of necrosis and non-viability in contrast to the non-stimulated, slower growing controls. The interpretation of depressed specific activities is thus open to some question. When consistently accelerated incorporation rates are obtained after standard hormone injections, however, there would appear to be little reason for doubting that such results indicate an increase in nucleic acid synthesis and very probably a concomitant acceleration in growth rate of the tumor.

With these general considerations in mind, the results recorded in Tables 1 to 4 may be summarized as follows:

1. Estrogen caused an acceleration of P^{32} incorporation into the mammary carcinoma of castrated females, using a total of 43 injected mice and 45 non-injected controls in 9 different experiments. No material difference was noted when the dose was increased from 2 to 10 gamma for 2 or 3 days before sacrifice.

2. Testosterone given to castrated females was associated with no change or in some cases a depression of this rate (Table 2).

3. When estrogen was given to castrated males with tumor transplants no clear-cut conclusions could be drawn (Table 3), but when testosterone was used (Table 4) a very definite stimulation was noted in five experiments using a total of 31 grafted mice and 26 controls, with the exception of one group of animals where a depression was recorded in association with advanced necrosis of the tumor.

The method of computing the specific activity for phosphorus in the nucleic acid fraction of these tumors has been found reliable in a considerable number of previous experiments from this laboratory.[2-4] This report suggests the value of this technique to demonstrate an effect of certain steroids on aspects of growth of some tumors of the reproductive tract that may be sensitive to changes in their steroid hormone environment.

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COMBINATION CHEMOTHERAPY OF CANCER BASED UPON QUANTITATIVE BIOCHEMICAL DIFFERENCES*

DANIEL M. SHAPIRO

So far as our present knowledge indicates, cancer tissues appear to possess the identical enzymes or other components that are known to be present in normal tissues The difference between the cancer cell and the normal cell is at present ascribed to alterations in relative amounts or activities of individual biochemical constituents, i.e, it would appear to be the overall metabolic pattern of the cancer cell that distinguishes it from the normal.

Although no single enzyme has yet been found whose activity in tumors is lower than that in the least active normal tissues, studies of individual enzyme systems indicate that, in general, tumors possess lower enzymatic activities than the majority of normal tissues. This is of pertinence to the problem of cancer chemotherapy in that no single normal tissue appears to possess the *identical over-all* complement of low enzymatic levels exhibited by cancer tissues. Therefore, sin
zymes present in low concentration in
summation at the cancer cell level with c
to a variable number of normal tissues.

The concept that it may be possible to antagonize an enzyme present in cancer tissue in small amounts, while producing only minimal effects on the same enzyme present in other tissues in larger amounts, has its origin in work done over the past fourteen years in many diversified fields of research by numerous investigators The mechanisms involved in anti-metabolite–metabolite phenomena are believed to revolve largely about quantitative relationships. To begin with, it is thought that the reaction between an enzyme and a metabolite results in the formation of an enzyme-metabolite complex, which in turn dissociates to produce the reaction product and the original enzyme. The anti-metabolite is considered to form a complex with the enzyme that will not dissociate into product and enzyme. The importance of quantitative relationships is depicted in Figure 1 employing the standard key and lock diagram. It is apparent that, other things being equal (e g, cellular permeability), whether or not inhibition occurs depends upon the representative concentrations of metabolite and antagonist molecules present

It is against this background of information, coupled with the general observation that many components (e.g, the B vitamins) are usually low in cancer tissue, that the hypothesis of employing quantitative biochemical differences as a basis for the selection of anti-cancer agents is advanced.[1-3] It would seem reasonable to expect such anti-metabolites, if uniformly distributed among the tissues of a tumor-bearing host in vivo, to result in greater adverse effects on the tumor than on the normal tissues. Furthermore, a multi-combination selected upon such a basis might cause sufficient

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simultaneous damage to so many metabolic pathways within the cancer cell as to result in rapid cell death before the development of drug resistance or drug dependence.

EXPERIMENTS

The suggested chemotherapeutic approach is under detailed appraisal by the Surgical Research Laboratories at Presbyterian Hospital by means of integrated microbiological, biochemical and biological studies in transplantable mammalian tumors. Initial studies were begun upon vitamin B_6 metabolism, since it had been reported in low concentration in most tumors[4] and because a potent antagonist, desoxypyridoxine, was available.

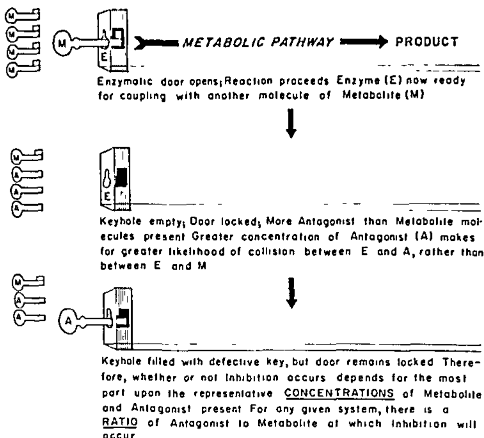


Fig 1. Importance of quantitative relationships in inhibition phenomena.

The vitamin B_6 content was found for the tissues of male C_{57} black mice as follows:[5] liver 5.28, kidney 4.16, heart 4.62, brain 2.84, muscle 2.70, stomach 1.50, testes 1.06, spleen 0 88, lungs 0 78, and the 755 transplantable mammary adenocarcinoma 0.69 micrograms per gram of wet weight. Thus, by far the majority of the normal tissues analyzed contained much higher concentrations of total vitamin B_6 than did the tumor, the only tissues falling close to the tumor being the testes and lungs. Administration of desoxypyridoxine to tumor-bearing mice produced a weak carcinostatic effect which was greatly bolstered by combination therapy with a guanine antagonist, 8-azaguanine.[3] More detailed study of the possible mechanisms involved revealed glutamic-aspartic transaminase, a vitamin B_6-containing

enzyme, to be in low concentration in tumor as compared to a number of normal tissues, and to be inhibited by desoxypyridoxine.[5] The results of studies on the inhibition of transaminase levels in a number of tissues demonstrated, as previously shown by Reif and Potter,[6] that the lower the initial enzyme concentration in a tissue, the greater the degree of inhibition by an antagonist to the enzyme[5] It therefore is understandable to find that a testosterone-produced decrease in the vitamin B_6 content of the 755 *tumor,[5] without any demonstrable effect on tumor growth,[7] permits suc-*

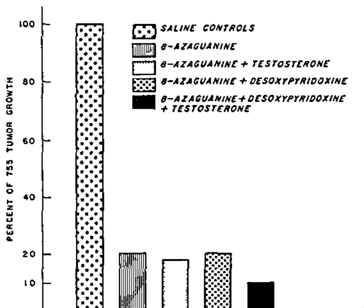


Fig 2 *All groups received 18 intraperitoneal injections.*

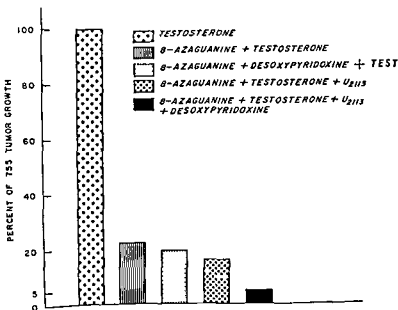


Fig. 3 All groups received 9 intraperitoneal injections.

cessful combination therapy employing doses of desoxypyridoxine otherwise too low to be carcinostatic when used alone.[5] Figure 2 summarizes the results of such a biological experiment.

These observations on vitamin B_6 metabolism in malignant tissue furnish evidence in support of the hypothesis that low metabolite concentrations render neoplastic cells vulnerable to appropriate anti-metabolite therapy. Similar studies on riboflavin metabolism are in progress.[8] The addition of a riboflavin antagonist, 6, 7-dimethyl-9-hydroxyethyl isoalloxazine, or U-2113, to the triple combination of 8-azaguanine plus desoxypyridoxine plus testosterone has produced greater carcinostatic results than any of the possible smaller combinations of three of these compounds. Figure 3 depicts graphically the results of such an experiment, the details of which have been given elsewhere.[8]

DISCUSSION

Two interesting observations arise from comparative study of Figures 2 and 3. The apparent lack of carcinostatic activity evidenced by the triple combination of 8-azaguanine plus desoxypyridoxine plus testosterone in Figure 3, as compared with the obvious carcinostatic activity of the same triple combination in Figure 2, must be viewed against the background of only nine injections for the experiment recorded in Figure 3. However, the observation that the quadruple combination in Figure 3 exerts even greater carcinostatic activity than the best efforts of the triple combination in Figure 2, becomes even more significant when viewed against the background of the number of injections. It took only half of the number of injections to achieve the still better carcinostatic results recorded in Figure 3. Space limitations preclude the delineating of toxicity data in the entire series of combination experiments evaluated to date. However, it should be stressed that as compounds have been used in multi-combination it has been found possible to achieve greater tumor inhibition with less host toxicity. This may have been due to the occurrence of synergism at the level of the cancer cell, thereby permitting smaller doses of each agent to be employed when used in combination. Thus, multi-combination chemotherapy would appear to "cross-fire" on the tumor cell, and to hit, only sparingly, a variable number of normal tissues. It seems, on the basis of the recorded data, that the greater the number of synergistic anti-metabolites successfully employed in a multi-combination, the greater may be the therapeutic index achieved

There are additional reasons to be considered for employing *multi*-combination therapy in the treatment of malignant disease. It is generally believed that spontaneous mutations occur in a cancerous cell population, and that these mutations are stable, irreversible and hereditable. An effective anti-cancer drug may exclude these mutant forms, resulting in a resistant or dependent tumor. If this reasoning be correct, then logic suggests that cancer chemotherapy should be completed quickly in order to successfully combat the spontaneous mutation rate. As indicated above, multi-combination chemotherapy may afford an opportunity to shorten the course of treatment.

Furthermore, since immune phenomena have not been demonstrated for human cancer, and indeed, seem unlikely, it may be necessary for the chemotherapy of cancer to be carcinocidal per se. Unlike the bacteria vs.

antibiotic combat, cancer drugs will probably have to achieve success without the aid of host antibodies, white blood cells or other resistant mechanisms The simultaneous blockade of multiple metabolic pathways in the cancer cell as a means of achieving a rapidly lethal effect seems a reasonable possibility.

The current emphasis upon chemotherapy as a potential method of treatment for malignant disease has been occasioned by the feeling that the two currently accepted forms of therapy, surgery and radiation, have reached their zenith and have been found wanting. However, it is doubtful if successful cancer chemotherapy, leaving behind large areas of dead cells, is feasible without concomitant surgery It is pleasant to envisage hopefully the eventual possibility of nonmutilating but effective cancer chemosurgery.

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ISOTOPE THERAPY FOR CARCINOMA OF THE PANCREAS*

P. V. HARPER AND K. A. LATHROP**

The radical surgical treatment of carcinoma of the pancreas has proven to be useless. For this reason a reexamination of the possibilities of local radiation therapy has been undertaken The radium volume implant technique developed by Paterson and Parker[1] produces in the treated region a relatively uniform localized field of radiation. The development and clinical trial of a flexible extension of this method form the basis for this report.

The use of radium, cobalt, or radon implants for intra-abdominal tumors

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has certain inescapable disadvantages. Plans for having the isotope available must be made well in advance of its use, hence, unexpected situations usually cannot be dealt with at the primary operation. The operator is subjected to substantial radiation dosage in the course of making the implantation and closing the abdomen. It is necessary to remove the implant at the termination of treatment (except for radon seeds), and the possibility of loss and the consequent dangerous search must be considered. Postoperative complications such as wound disruptions, which require surgical intervention, can lead to very uncomfortable situations for the surgeon.

TECHNIQUE

In order to circumvent these difficulties, the following technique was devised. Very fine polyethylene tubing (Clay Adams PE-10, outer diameter 0.61 mm., inner diameter 0.28 mm.), which is kept sterile and available at all times in the operating room, is threaded around and through the tumor, following as closely as possible the Paterson-Parker distribution rules, and subsequently filled with γ emitting isotope in solution. Three quarters of the tubing is evenly distributed on the surface of the tumor and one-fourth is placed evenly through the volume. The tubing is spaced about 1 cm. between turns, and careful measurements are made of the length of tubing comprising the implant and of the ends leading from it. The ends of the tubing are brought out through the wound and left long, one end being longer than the length of tubing in the implant. During this process the tumor is disturbed and handled as little as possible. The tubing may be filled with mercury, and the distribution checked by x-ray before closing the abdomen. This permits careful, painstaking placement of the implant without
organs may be
extent is feasible
tion, roentgenograms of the implant with mercury in the tubing are made, and from these, the volume of tissue to be irradiated is calculated.

I^{131} is used as a source of γ rays. The tubing wall filters out a portion of the β radiation, and the tissue immediately adjacent to the tubing filters out the rest. This tissue is exposed in any case to a very intense γ ray field so that little additional damage is done by the β radiation. Radioactive iodine was chosen because it is cheap, readily available, and has suitable chemical properties for concentration to the necessary small volume, and because the short half-life made removal of the isotope unnecessary.

The air dose rate at 1 cm from a 1 mc point source of I^{131} is 2.25 r per hour[2] as compared with 8.3 r per hour for 1 mg of radium. Thus, 3.7 mc. hours of I^{131} correspond to 1 mg. hour of radium. The linear absorption coefficients in tissue for radium and I^{131} γ rays are both about 3 per cent per centimeter,[2] so that this same proportion should be valid at various distances from the sources in tissue. On this assumption, 1 mc. of I^{131}, allowed to decay completely, gives the same tissue dose as 75 mg. hours of radium. Using this factor, the Paterson-Parker radium tables may be used directly to calculate the number of mc. that are needed to produce the desired total dose of radiation.

A quantity of I^{131} that is about 20 per cent in excess of the required amount, in a 5 to 10 cc. volume, is placed in a conical centrifuge tube. One or 2 mg. of KI are added as carrier, and the iodine is precipitated as AgI

with a 50 per cent excess of $AgNO_3$. The solution is acidified with HNO_3, digested at 70° for an hour to flocculate the precipitate, and then centrifuged. The supernatant is decanted, and the precipitated AgI is dried with a stream of air in the dark at room temperature. The supernatant is surveyed to check which is usually about 99 per cent. ope is handled at all times in a lead- . The total-body exposure to the operator, determined by a pocket dosimeter during the processing of 200 mc. of I^{131} without taking extraordinary precautions, was 7 mr.

Silver iodide is prepared for placement in the implant by dissolving it in a sufficient volume of saturated KI to give the correct number of millicures per cm. of tubing. This is accomplished with sufficient accuracy by filling an appropriate length of the polyethylene tubing with saturated KI and discharging it onto the AgI precipitate, which dissolves readily.

The isotope is then brought to the patient's bedside. The tubing that has

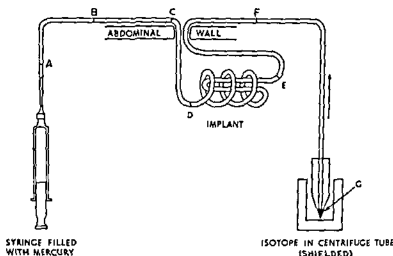


Fig 1 *Diagram showing method of placement of isotope in tubing (see text).*

been implanted is cleaned with distilled water dried with alcohol, ether, and air and filled with clean, dry mercury.

The tubing is marked as shown in Figure 1, where $AB = CD$, $DE = FG$. The distance DE is measured at the time of surgery, and CD is the difference between AD, measured at surgery, and AC. The long end of the tubing, on which the distance FG is marked, is placed in the centrifuge tube containing the isotope solution, which is drawn up by suction into the tubing until the solution reaches point F. The end of the tubing is then withdrawn from the centrifuge tube, and the isotope solution is drawn into the implant until the head of the column of isotope solution appears at point C beyond the tumor. The syringe is then removed from the tubing and the mercury is allowed to drop back to point B, forcing the isotope solution back to point D. When this procedure is carried out properly, the isotope is in the tubing within the tumor while the two leads going down to the implant contain air or mercury. The ends of the tubing are sealed with heat and pressure and left long. This entire procedure usually takes about a minute, and exposure to the operator is of the order of 10 mr.

If it is necessary to re-explore the patient, the isotope may be removed and later replaced without difficulty. Similarly, the isotope can be removed prior to autopsy.

The tubing is left in place after the decay of the isotope. The ends are cut short, sealed, and allowed to retract beneath the skin, where they cause no difficulty.

CLINICAL RESULTS

This procedure has been attempted successfully in 6 cases.

1. L.M., Number 58-65-08. This 55 year old woman had a mass in the pancreas discovered 6 months previously at cholecystectomy. She was re-explored at this hospital on November 5, 1953. At operation, a large tumor about 135 cc. in volume, in the head and body of the pancreas, was implanted with tubing. It was impossible to place the core of the implant symmetrically because the superior mesenteric artery and portal vein traversed the implant. The common duct was transplanted to the greater curvature of the duodenum, and the pylorus was transected and an anterior gastroenterostomy was performed. These procedures removed the bile duct and stomach from the field of irradiation. One week after operation 135 mc. of I^{131} were placed in the tubing, giving a calculated dose of 5000 r to the lowest point in the implant. The patient tolerated this well, without evidence of radiation sickn... isotope administration she developed ... and leukopenia (white count 1900). ... transfusions and supportive therapy. The patient's epigastric pain and back pain were much improved so that she was able to discontinue completely the use of narcotics. Her ... tanned from radiation ... to fail. Numerous ab- ... jaundiced and died on June 8, 1954. No autopsy was obtained.

2. M.S., Number 58-90-08. This 66 year old woman had a history of back pain, weight loss, and anorexia for 9 months and had a moderate obstructive jaundice for 2 weeks. She was explored on December 5, 1953, and a small tumor (15 gm) of the pancreas or distal common duct was implanted with tubing. The common duct was transplanted to the greater curvature of the duodenum to relieve the obstruction. On December 10, 10.6 mc. of radioactive iodine were placed in the tubing, and 5 days later, this was removed and replaced with 21.3 mc., which gave a calculated radiation dose of 10,700 r The patient's jaundice disappeared but she continued to have some pains in her back. She did fairly well for 3 months. There was no apparent adverse effect from the radiation. She was hospitalized again on January 9, 1954, when she complained of nausea and vomiting. Roentgenograms of the duodenum adjacent to the implant revealed no abnormal condi- ... The patient expired on March 23, 1954. ... to chronic pyelonephritis. Grossly, there ... pic studies revealed that tumor tissue was infiltrating the peripancreatic tissues In the region of the implant, the tumor showed marked regression. There was some evidence of fibrosis in the duodenum adjacent to the implant.

... 5 month history of abdominal ... nination was not remarkable. ... Roentgenograms were negative. On exploratory laparotomy February 1, 1954, a carcinoma was found that involved almost the entire pancreas and the surrounding tissue There was some local lymph node involvement and a few small metastases to the under surface of the liver. A gastrojejunostomy, cholecystoduodenostomy, and splanchnicectomy were done, and the pancreas was implanted with polyethylene tubing The patient had an uneventful recovery. On February 11, 1954, 176 mc of iodine were placed in the tubing. The radiation dose in the implant was difficult to estimate because of the peculiar elongated shape of the tumor. The volume was estimated as about 150 cc, and the radiation dose at about 10,000 r. The patient was discharged on February 19, 1954, and returned to work 3 weeks later. His back pain was nearly gone, and his only complaints were persistent diarrhea and inability to gain weight. Four weeks after the isotope administration he

developed marked leukopenia and anemia, which required no particular treatment and regressed spontaneously. He continued to do well until about May 1, 1954, when back pain recurred He expired suddenly on June 19, 1954. An autopsy revealed marked destruction of the pancreas in the region of implant, without significant damage to any nearby structures. The portal vein was patent The tumor, however, had spread extensively to the liver and to the gastrocolic omentum. No anatomic cause of death was found.

4 J B, Number 60-10-62 This 59 year old man complained of severe burning abdominal pain for 1 year and had lost 25 pounds in the 6 months prior to admission. Physical examination was otherwise negative and roentgenograms revealed a pyloric lesion of undetermined nature On May 21, 1954, the patient was explored and a tumor mass of about 120 cc in volume was found in the head of the pancreas. Anterior gastrojeju-



Fig 2. X-ray of typical implant (case 6).

of further spread or radiation damage to other organs. The fistula from the jejunum communicated with the implant, which was infected, and the cause of death was a septic thrombophlebitis of the portal vein

5 T.D, Number 60-43-91. This 49 year old woman had a painless obstructive jaundice for 6 weeks On July 2, 1954, she was explored and a carcinoma of the distal common duct was found to be causing the obstruction. A gastrojejunostomy and cholecystjejunostomy were performed, and the tumor mass was implanted with polyethylene tubing The volume of the tumor was calculated to be 33 5 cc. The patient made a rapid recovery, and on July 8, 1954, 32 3 mc. of iodine were placed into the tubing. She has

had no adverse effect from the radiation and continues to do well and has been gaining weight. The total dose delivered to the tumor was 8300 r.

6 T.R., Number 60-97-75. This 73 year old man had a typical history of painless obstructive jaundice for 8 months. In August of this year he was explored at another hospital and a tumor approximately 25 cc. in volume was found in the head of the [illegible] plant of polyethylene tubing was [illegible]

DISCUSSION

It appears from these observations that radiation may be delivered to intra-abdominal tumors in amounts that are intolerable to the patient if the same dose were delivered externally. This is probably because of the relatively small volume of tissue irradiated, and because it is possible to move vulnerable organs away from the field of radiation. In all the cases in which pain was a problem, some relief was achieved. Cases 3 and 4 were done unexpectedly without prior planning. The great need for being able to do the implantation at the primary exploration is illustrated by one case (not abstracted) in which the operation was rendered technically impossible by extensive reaction from the previous surgery. It seems feasible at the present time to attempt to deliver cancericidal doses to early small carcinomas of the pancreas. Radiation in this form seems to be well tolerated and the results from such treatment can certainly be no worse than those from radical surgery. Small implants with dose levels ranging from 20,000 to 40,000 r are being made in experimental animals to investigate the dangers and limitations of the application of this method.

The use of isotope other than I^{131} is also being explored and will form the basis for later reports.

SUMMARY

An extension of the conventional radium implant technique is presented that permits much more flexible application of the method to include the use of various artificial radioactive isotopes.

Clinical experiments using this method are yielding encouraging results.

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ANTIBODIES TO CANCER IN PATIENTS*

JOHN B. GRAHAM AND RUTH M. GRAHAM

Cancer is widely regarded as an autonomous process whose growth rate and course are determined solely by the virulence of the tumor. This concept *further provides that 100 per cent removal or immediate destruction* of the tumor is essential for long-time survival of the patient.

However, there are some observations that fail to agree with this thesis that suggest the presence of host resistance. The development of late recurrence after a period of several years' apparent freedom from the disease, especially in association with intercurrent disease, is more likely to be the result of a more favorable environment than of a change in the aggressiveness of the cancer cells. The presence of cancer cells in the clean operative field at the end of operation and in the postoperative period occurs frequently and apparently is not inimicable to the subsequent "cure." The latter observation has been made in radical mastectomy[1] and radical hysterectomy[2] Cancer elicits an inflammatory response of varying intensity and is seen with such regularity in some sites, e.g., the uterine cervix, that, *when absent, the diagnosis of malignancy may be suspect. The degree of* inflammatory response may correlate with the prognosis in selected lesions of the stomach. Steiner et al.[3] have shown that patients who survive 5 years have more pronounced inflammation about the tumor than those who succumb promptly

The search for antibodies to cancer has continued for half a century with indifferent success. Part of the difficulty stems from too close an analogy with infectious diseases in that a common antigen has been used as the testing agent On consideration, it seems likely that the immunologic differences between individuals probably far exceed the difference between a spontaneous tumor and the individual who harbors it With the latter concept in mind, a renewed look at this problem has been initiated

METHODS

Patients have been tested with antigen extracted from their own tumors, by the complement fixation technique The antigen is prepared by mechanically fragmenting the tumor obtained from the surgical specimen or biopsy until no intact nuclei remain A water-soluble, saline-insoluble fraction is obtained that is largely desoxynucleoprotein, but also contains other substances in less amount The antigen must be prepared aseptically and at a low temperature (5°C.) in order to minimize alteration of the proteins. It has been difficult to obtain large enough amounts of tumor to work with comfortably. Usually no more than 1 to 2 grams and, rarely, 4 to 5 grams of strictly tumor tissue can be procured from a clinically favorable case Blood drawn before and after treatment has been tested.

Forty-eight patients with cancer have been tested in this manner. The sites of the lesions are as follows. cervix, 31, ovary, 6, uterine corpus, 5, vulva, 3; vagina, urethra, and breast, each 1.

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RESULTS

Of the 48 cases, 12 were found to have complement fixing antibodies to their tumor in a titer of 1:16 to 1:128.[4] Nine of those with positive titers had a malignancy arising in the cervix, two in the vulva, and one in the ovary. The antigen preparations varied a good deal in their concentration, as indicated by the level of nitrogen. In 30, the nitrogen concentration ranged from 0.1 to 6 mg. per cent; in one it was 15 mg. per cent; and in another, 18 mg. per cent; the remaining 16 had a trace or less.

The preparations containing less than 0.1 mg. per cent nitrogen are referred to as weak antigen, and those with a concentration of more than 0.1 mg. per cent nitrogen are called strong antigens. Only one of the 16 patients tested with a weak antigen showed specific antibodies and that preparation contained a trace of nitrogen. Of those 32 with a strong antigen, circulating antibodies could be demonstrated in 11. This striking difference in the apparent frequency of complement fixing antibodies is felt to be a reflection of the adequacy of the testing antigen, for if it is too dilute the antigen may be incapable of demonstrating antibodies, even though present. This experience suggests that a preparation of antigen by this method should have 0.1 mg. per cent nitrogen or more in order to test the serum adequately.

Table 1. Site of Tumor, Distribution of Cases, and Frequency of Antibodies to the Cancer

	NUMBER OF CASES	
SITE	TOTAL	WITH DEMONSTRABLE ANTIBODY
Cervix	31	9
Ovary	6	1
Corpus	5	0
Vulva	3	2
Vagina	1	0
Urethra	1	0
Breast	1	0

The cases studied have been divided into two clinical groups, "favorable" and "poor." Those called "favorable" had a primary lesion that was operable, or probably curable by radiation. Those called "poor" included recurrent, inoperable or extensive cases unlikely to be cured by irradiation. There were 20 "poor" cases, 16 of whom had an antigen with measurable nitrogen, and 28 "favorable" cases, of whom 16 had measurable nitrogen in the antigen. The lower incidence of measurable nitrogen in the antigen of the favorable cases reflects the smaller size tumor in that group. For although it is rather easy to get several grams of tissue in the advanced, inoperable case, the procurement of even a single gram of tumor in the clinically favorable case may be impossible.

There is a pronounced difference in the frequency with which specific antibodies can be demonstrated in the two clinical groups. Of the 20 cases classified as poor, only 2 (10 per cent) had demonstrable antibodies. By contrast, of the 28 clinically favorable cases, 10 (36 per cent) showed a significant titer. The difference is even more distinct in those tested with a

strong antigen, for there, 2 (12 per cent) out of 16 clinically poor cases and 9 (56 per cent) of 16 favorable cases had demonstrable antibodies. One of the cases called "poor" had an inoperable cancer of the ovary subsequently treated with x-ray. Five observations over 11 weeks demonstrated anti-tumor effect on only one occasion, that one week after x-ray was completed. She obtained a remission from this treatment that lasted almost a year. The other "poor" case had a cervical carcinoma treated with exenteration that apparently removed all the tumor, however, the patient died 9 months later of recurrence.

The preponderant concentration of the demonstrable antibodies in the clinically favorable cases rather than in the cases with more extensive tumor is as one might expect, for the patient who is under the shadow of impending defeat is less likely to show evidence of resistance than one who is holding her tumor in check.

Serial observations are not available on all of the cases. Some were tested only prior to treatment, others only after treatment, while the remainder had sera drawn both pre- and post-treatment for testing. Two patients had pretreatment titers and no subsequent tests. Ten were observed to have

Table 2. Forty-eight Cases Tested for Antibodies to Their Own Tumor

WEAK ANTIGEN (LESS THAN 0.1 MG. % N)			STRONG ANTIGEN (MORE THAN 0.1 MG % N)		
CLINICAL STATUS		POSITIVE TITER	CLINICAL STATUS		POSITIVE TITER
Favorable	12	1	Favorable	16	9
Poor	4		Poor	16	2
Total	16	1	Total	32	11

circulating antibodies after treatment. Six of these were studied prior to treatment and, although 3 showed a titer, 3 did not, which could be explained by the prompt fixation of antibodies by the tumor when it was intact but, once removed, the antibodies produced would tend to accumulate in the blood stream, as there was no tumor to react with them.

An example case follows. AR, 794522, age 44, had a squamous carcinoma of the cervix, stage I, treated by radical hysterectomy and regional lymphadenectomy The nodes were negative. The antigen contained 2.5 mg. per cent N. Serum taken 1 day preoperatively had an antibody titer of 1·32, 8 weeks postoperatively it was 1.128, 9 weeks postoperatively 1·64, and 16 weeks postoperatively 1·16. She is living and well now, two years later.

SUMMARY AND CONCLUSIONS

Forty-eight patients have been tested by complement fixation for antibodies to their own cancer. An antigen was prepared from each tumor by extracting the water-soluble, saline-insoluble fraction. Asepsis and gentle chemical handling are regarded as essential.

Of the 48 cases studied, 12 were found to have anti-tumor antibodies. These specific antibodies were found five times more frequently in clinically favorable patients than in those whose clinical outlook was poor. The

antibodies demonstrated here are regarded as a manifestation of resistance toward the tumor.

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INCIDENCE OF CARCINOMA IN THYROID GLANDS REMOVED AT 1000 CONSECUTIVE ROUTINE NECROPSIES*

J. D. MORTENSEN, WARREN A. BENNETT, AND LEWIS B. WOOLNER

To those interested in surgery of the thyroid gland the current controversy concerning the incidence of carcinoma of the thyroid is particularly disturbing. It seems evident that a conclusive answer to the question, "How common is cancer of the thyroid gland?" although certainly desirable, is not now available. We have endeavored, therefore, to investigate this question in a manner which to us appears to be impartial and rather definitive.

MATERIALS AND METHODS OF STUDY

The thyroid gland was removed in toto from each of 1000 patients examined at consecutive routine necropsies in the Section of Pathologic Anatomy of the Mayo Clinic between July, 1951, and June, 1953 The glands were examined grossly in the routine manner by the pathologists performing the necropsies and were then turned over to us for this study. No attempt was made to select or screen the patients or the glands in any manner.

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a semiautomatic slicing machine which produced slices 2 mm. thick Each slice was inspected for nodules and a sketch was made for each gland, showing the number, size, distribution, and gross characteristics of all nodules or any other lesions that were so discovered Sections for histologic study were taken from parenchymal tissue and from representative nodules in every gland, staining was done in the routine manner by the hematoxylin and eosin technique. This pathologic examination was comparable in thoroughness to the examination of a surgical specimen of thyroid tissue made in a surgical pathology laboratory.

Findings on clinical and laboratory examinations and at necropsy were reviewed. Finally, microscopic examination of all histologic sections was carried out independently by each of the authors, one (W.A.B.) of whom usually examines necropsy specimens and another (L.B.W.) of whom customarily studies surgical specimens. According to the histologic findings, each nodule was classified first as either neoplastic or non-neoplastic; then the specific diagnosis of the nodule was made. A diagnosis of malignancy was not made unless all examiners agreed that the lesion met the commonly accepted criteria for malignant disease of the thyroid

* From the Mayo Clinic and Mayo Foundation, Rochester, Minnesota.

FINDINGS

Of the 1000 consecutive thyroid glands examined as described, 525 (52.5 per cent) were found to contain grossly recognizable nodules. Incidence of nodularity was greater in thyroid glands from women than in those from men, 68 per cent of the former and 44 per cent of the latter containing nodules. Regardless of sex, incidence of nodularity increased with age. A solitary nodule was present in 119 thyroid glands, which represented 12 per cent of all glands examined and 23 per cent of the nodular glands. A solitary nodule which was palpable to the examiner at necropsy with the gland actually in his hand, however, was found in only 26 thyroid glands, this number being 2.6 per cent of all glands and 5 per cent of the nodular glands.

Table 1. Malignant Involvement of Thyroid Glands Removed at 1000 Consecutive Routine Necropsies

TYPE OF MALIGNANCY OF THYROID	NO	PER CENT INCIDENCE IN ALL 1000 GLANDS	PER CENT INCIDENCE IN 525 NODULAR GLANDS
Primary carcinoma	28	2.8	5.3
Metastatic involvement	18	1.8	3.4
Total	46	4.6	8.7

Malignant neoplasms were demonstrated in 46 thyroid glands (Table 1), 18 of them being metastatic lesions and 28 primary malignant neoplasms. The incidence of primary malignant neoplasms of the thyroid, therefore, was 2.8 per cent in the 1000 consecutive routine necropsies, or 5.3 per cent in the 525 nodular thyroid glands.

The distribution of these primary thyroid carcinomas according to the age and sex of the patients (Table 2) indicates that although nodules develop more commonly and at an earlier age in women than in men, the

Table 2. Relationship of Age and Sex of Patient to Incidence of Primary Carcinoma of the Thyroid

AGE, YR.	SEX	CANCER	IN ALL PATIENTS: GLANDS	IN ALL PATIENTS: CARCINOMA, PER CENT	IN PATIENTS WITH NODULAR GLANDS: GLANDS	IN PATIENTS WITH NODULAR GLANDS: CARCINOMA, PER CENT
0-19	M	0	44	0	4	0
	F	0	26	0	5	0
20-49	M	1	151	1	33	3
	F	3	88	3	40	8
50-69	M	12	297	4	160	8
	F	5	135	4	102	5
>70	M	3	163	2	94	3
	F	4	96	4	87	5
All ages	M	16	655	2	291	5
	F	12	345	4	234	5
Total		28	1000	3	525	5

incidence of malignancy in nodular glands is not particularly related to the sex of the patient or, surprisingly, to his age so long as he is an adult.

The relationship of number and palpability (which depends on size, consistency, and location) of the nodules to the incidence of carcinoma (Table 3) suggests that if a nodule is solitary, it makes little difference whether or not it is palpable insofar as the likelihood of its being cancerous is concerned. A solitary nodule, regardless of its palpability, is malignant in about 9 per cent of the cases, this being two and a half times the incidence of malignancy in multinodular thyroid glands. It also was interesting to note that the incidence of malignancy in multinodular glands with a single palpable nodule was about three times greater than in multinodular glands with no palpable nodules or with several palpable nodules.

Table 3. Relationship of Number and Size of Nodules to Incidence of Primary Carcinoma of Thyroid

NO. OF NODULES	PALPABILITY	GLANDS	CARCINOMAS	INCIDENCE OF CARCINOMA, PER CENT
Solitary	Solitary nonpalpable	93	8	9
	Solitary palpable	26	3	12
	All solitary nodules	119	11	9
Multiple	Multiple nonpalpable	202	7	3
	1 palpable but also multiple nonpalpable	66	6	9
	Multiple palpable	138	4	3
	All multiple nodules	106	17	4
All nodular glands		525	28	5

Table 4. Pathologic Classification of Primary Thyroid Malignant Lesions Found in 1000 Consecutive Necropsies

	NO.	PER CENT
Low-grade carcinomas		
Papillary carcinoma	15	54
Follicular carcinoma	6	21
Solid carcinoma	3	11
Hurthle cell carcinoma	1	3
High-grade carcinomas		
Anaplastic carcinoma	3	11
Total	28	100

Pathologic classification of the histologic type of primary carcinoma found in the thyroid gland (Table 4) parallels rather well the findings in carcinoma of the thyroid found in surgically removed nodular goiters. Most of these occult carcinomas of the thyroid were of the low-grade variety, about 55 per cent being papillary carcinomas. Four of these papillary carcinomas resembled the relatively benign "non-encapsulated sclerosing papillary tumors," which have been shown capable of metastasis. There were, however, 3 glands containing high-grade anaplastic carcinomas, 2 of which were

occult, not being recognized either clinically or during routine postmortem examination.

The following important findings (Table 5) in our opinion, explain some of the controversy concerning the incidence of carcinoma of the thyroid gland: (1) of the 28 primary carcinomas of the thyroid, only 1 was recognized while the patient was living, (2) only 1 of the 1000 patients died from carcinoma of the thyroid, this being the same patient who had clinically recognized carcinoma of the thyroid, and (3) in only 2 of the 1000 glands was a malignant lesion of the thyroid recognized at routine postmortem examination. All this would seem to indicate, as has been reported, that malignant disease of the thyroid gland is indeed unusual. Yet, when these same thyroid glands were examined carefully, all grossly recognized nodules being studied histologically, 26 additional occult, but real, cancers were

Table 5. Recognizability of 28 Primary Carcinomas of Thyroid Detected in 1000 Routine Necropsies

	NO.	INCIDENCE, PER CENT
	1	0 1
	1	0 1
routine necropsy	2	0 2
Occult carcinoma of thyroid	26	2 6

discovered, constituting an actual rate of incidence of thyroid cancer of 2 8 per cent in the 1000 consecutive routine necropsies and 5 3 per cent in the nodular thyroid glands.

COMMENT

The relatively high incidence of occult carcinoma of the thyroid gland is not irreconcilable to the admitted infrequency of carcinoma of the thyroid as a cause of death. It simply demonstrates that carcinoma of the thyroid in many patients is slow growing and that the patient dies from other disorders while harboring the malignant lesion. Such circumstances cannot be construed to indicate that carcinoma of the thyroid is innocuous and that it can be ignored clinically, for it must be realized that the chance for cure of any carcinoma, including that of the thyroid gland, is greatest when the lesion is localized, and, at the moment, innocuous.

The present unfortunate controversy over the actual incidence of carcinoma of the thyroid gland appears to be based first on routine necropsy findings and second on statistical manipulation without factual basis. The former we have demonstrated to be inadequate for detection of occult malignant disease of the thyroid, and the latter is hardly as reliable as actual pathologic findings.

SUMMARY AND CONCLUSIONS

Minute examination of thyroid glands removed in the course of 1000 consecutive routine necropsies at the Mayo Clinic has demonstrated that primary malignant disease of the thyroid gland is not rare. Indeed, its rate of incidence among patients coming to necropsy without clinically suspected carcinoma of the thyroid but with pathologically identifiable nodules

in the thyroid gland is about the same as the incidence of carcinoma repeatedly reported in clinically recognized and surgically removed nodular thyroid glands, that is, 5.3 per cent.

QUANTITATIVE MEASUREMENT OF BLEEDING FROM ALIMENTARY TRACT BY USE OF RADIOCHROMIUM-LABELED ERYTHROCYTES*

CHARLES A. OWEN, JR., MILTON COOPER, JOHN H. GRINDLAY, AND JESSE L. BOLLMAN

Conventional techniques for estimation of blood in feces depend on production of a characteristic color when certain chemicals, such as benzidine, guaiac or orthotolidine, are mixed with the feces and hydrogen peroxide is added. Unfortunately, the ingestion of meat is usually sufficient to induce positive results in such color tests in normal stools.

[illegible]

two elements has been reported, namely radiochromium (Cr^{51})[1] and radio-

Table 1. Comparison of Guaiac Test and Cr^{51}-labeled Erythrocyte Method for Estimating Blood in Feces

ML. BLOOD PER 100 GM. FECES	MG. HEMOGLOBIN PER GM. FECES	GUAIAC TEST	ML. BLOOD PER 100 GM. FECES, CR^{51} METHOD
10	12.80	Positive	9 7
2	2.56	Positive	2.5
1	1.28	Positive	*
0 5	0 64	Weakly positive	*
0.25	0 32	Doubtful	*

* Insufficient Cr^{51} to distinguish reliably from background.

iron (Fe^{59}).[2] Since erythrocytes can be bound with chromium in vitro, we have used only this element.

METHODS

Guaiac Test. To compare the absolute sensitivity of the guaiac and radiochromium methods, Cr^{51}-labeled blood was drawn from a patient who had received about 250 microcuries (μc.) of Cr^{51} (bound to his erythrocytes) 1 week previously. The blood, which contained 12 8 gm. of hemoglobin per 100 ml., was added in various proportions to a specimen of human feces that had given negative results with both guaiac and benzidine chemical tests. The results are shown in Table 1.

It is apparent that with concentrations of Cr^{51} such as were routinely used

* From the Mayo Clinic and Mayo Foundation, Rochester, Minnesota.

in patients, the radioactive method was less sensitive than the guaiac test. However, the guaiac test often gives positive results in normal persons ingesting normal diets, and precise quantitative techniques are not available. The radiochromium method cannot be influenced by the diet, and it does permit reasonably precise quantitative measurement; the sensitivity was satisfactory down to a value of about 1 to 2 ml. of blood per 100 gm. of feces.

Radiochromium Method. Freshly drawn blood was mixed promptly with heparin for studies in dogs; ACD solution (citric acid-sodium citrate-dextrose) was used for studies in humans. Radiochromium in the form of sodium chromate* was added to the blood. In the case of patients, sterile precautions were observed throughout. A sample of the blood was tested for the efficiency of erythrocytic tagging according to the following formula (labeling was stopped by the addition of ascorbic acid, which converts the chromate ion to the chromic form, the latter cannot be bound to erythrocytes):

$$\frac{Cr^{51} \text{ in erythrocytes from } 1.0 \text{ ml. blood}}{Cr^{51} \text{ in } 1.0 \text{ ml. whole blood}} \times 100 = \text{per cent of labeling.}$$

When ACD solution was used as an anticoagulant, the labeling efficiency was usually about 90 per cent. Therefore, it appeared to be unnecessary to remove the unbound Cr^{51} moiety by centrifugation and washing of the cells.

A specific example of the labeling technique used for a patient follows. About 50 ml. of blood was withdrawn and immediately placed in a sterile bottle containing 10 ml. of ACD solution to which had been added 0.60 ml. of a solution of sodium chromate, representing 306 microcuries of Cr^{51}. The bottle was rotated slowly for a period of 15 minutes in a water bath at 37°C. Exactly 40 ml. of the ACD-blood-Cr^{51} mixture was then returned intravenously to the donor. The dose of Cr^{51} was $\frac{40}{50 + 10 + 0.6} \times 306 =$ 202 microcuries of Cr^{51}. The remainder of the blood in the bottle was set aside for standards. To 8 ml. of the ACD-blood standard was added 2 ml. of 1.25 per cent solution of ascorbic acid in saline. The radioactivity of 1.0 ml. of this mixture was recorded as 5568 counts per second in a sodium iodide well counter. The erythrocytes from this 1.0 ml., after being washed thrice with saline solution, yielded 5062 counts per second. Therefore, the labeling efficiency was $\frac{5062}{5568} \times 100 = 91$ per cent. The actual dose of Cr^{51} attached to erythrocytes was $202 \times 0.91 = 184$ microcuries. Anaerobic and aerobic cultures were made of some of the residual blood; without exception these have been negative for bacteria.

Fecal collections were begun after injection of the Cr^{51}-labeled blood into the patient or animal. The feces were homogenized with added water and the gamma rays of Cr^{51} were measured in weighed aliquots of the homogenized material. For small fecal samples, we used a thallium-activated sodium iodide well crystal; for larger specimens (about 60 gm.), we employed a Texas Co. well counter of the Geiger type. At our routinely used settings, the sodium iodide crystal yielded about 1400 counts per second per microcurie, this is a satisfactory figure when it is considered that Cr^{51} emits

* Obtained from Abbott Laboratories under authorization of the Atomic Energy Commission, specific activities were about 1 millicurie (mc.) per milligram.

gamma rays with only about 8 per cent of its disintegrations and that 1 microcurie is 37,000 disintegrations per second. The Texas Co. well counter had a sensitivity of about 60 counts per second per microcurie of Cr^{51}. The radioactivity of an entire fecal specimen was calculated from the aliquots counted. This value was then compared with the radioactivity of the circulating blood obtained the day the stool was passed, according to the following formula:

$$\frac{Cr^{51} \text{ in 24-hour fecal specimen}}{Cr^{51} \text{ in 1 ml. of circulating blood}} = \text{ml. of "blood" (as } Cr^{51}\text{) in the feces per day.}$$

Since the radioactivity of the circulating blood changed little from day to day, it was not necessary to draw a new specimen of blood daily, particularly in the more prolonged studies.

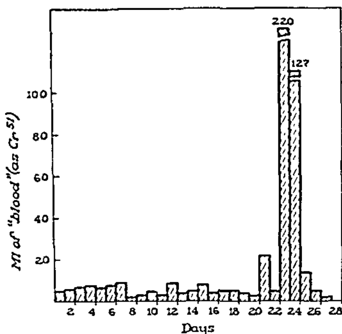


Fig. 1. Calculated loss of blood in feces as measured by radiochromium-labeled erythrocytes. Note the explosive hemorrhage on the twenty-third day.

RESULTS

In normal dogs[1] it was found that the amount of Cr^{51} present in the feces each day was comparable to the Cr^{51} contained in about 1 ml. of circulating blood. It was considered that only a small portion of this fecal Cr^{51} actually represented blood, the majority probably reflecting free chromium ions that had penetrated the intestinal wall.

At intervals of a few days amounts of blood were withdrawn from dogs previously "labeled" with Cr^{51}; measured amounts of this blood were promptly returned by gastric tube to the same dog to simulate gastric hemorrhage. The amount of Cr^{51} detected in the feces during the subsequent 24 to 48 hours approximated that given in the labeled blood. With amounts of more than 5 ml., the calculated "hemorrhage" was within 10 per cent of the volume of blood given. Because of the meat diet consumed by the dogs,

their feces gave positive results with the guaiac test whether or not blood was present.

Figures 1 and 2 represent the findings in 2 patients, both of whom had portal cirrhosis and esophageal varices. Both were anemic and had shown evidence in the past of bleeding from the alimentary tract. In Figure 1, the patient had a base line of 5 to 10 ml. of "blood" in the feces each day; some of these values appear to be slightly increased above the normal excretion of chromium. As in the dog, there was evidence of some Cr^{51} in the feces of normal persons whose erythrocytes had been labeled with this radioisotope, the normal excretion of Cr^{51} amounting to the equivalent of as much as 5 ml. of "blood" per day. Thus, only when the fecal Cr^{51} was significantly

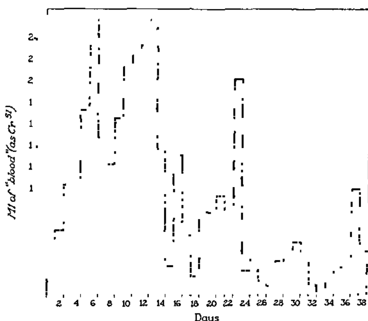


Fig 2 Calculated loss of blood in feces as measured by radiochromium-labeled erythrocytes in a patient who was actively bleeding Note the cyclic nature of the bleeding

greater than this could one claim the presence of actual blood. On the twenty-first day a definitely abnormal value of 22 ml. was obtained in this patient's feces; this was followed 2 days later by an explosive hemorrhage, presumably from an esophageal varix.

The patient whose findings are represented in Figure 2 was bleeding actively when first seen, the stools were black and oily. It is interesting to note the cyclic form of bleeding in this patient, each cycle lasted slightly more than a week and each, fortunately, tended to be less severe than the previous one. The patient's circulating hemoglobin began a steady spontaneous increase as the gastro-intestinal bleeding subsided.

COMMENT

The fact that only small amounts of radiochromium normally appear in the feces when the circulating erythrocytes are labeled with this radioactive element facilitates the quantitative measurement of gastro-intestinal bleeding.

The procedure is much more tedious than simple chemical tests, for the circulating blood must be labeled by use of a sterile technique, and an interval of 1 to 2 days must elapse to permit complete passage of unlabeled feces. However, there appears to be a place for this procedure when quantitative measurement of bleeding is of importance. We also have found it of use in those patients in whom the labeled erythrocytes disappeared from the circulation at an excessive rate; only when gastro-intestinal or other sources of hemorrhage could be excluded did this finding warrant the impression of an intrinsic hemolytic process.

CONCLUSION

The appearance of significant amounts of radiochromium (Cr^{51}) in the feces, after the labeling of the circulating erythrocytes with this radioactive isotope of chromium, affords a qualitative and quantitative method for the evaluation of bleeding from the alimentary tract.

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OBSERVATIONS ON THE ROLE OF THE LIVER IN THE METABOLISM OF STEROID HORMONES IN PATIENTS WITH ADVANCED METASTATIC BREAST CARCINOMA*

MAURICE GALANTE, J. MAX RUKES, MARY E. FLANAGAN, PETER H. FORSHAM, AND DAVID A. WOOD

The elimination of estrogens has long been known to be beneficial in the palliative treatment of some types of metastatic carcinoma of the breast. Bilateral oophorectomy was first shown by Beatson[1] to induce temporary remissions in the growth of breast carcinoma. Subsequent work by Huggins[2] demonstrated that further improvement could be produced by the removal of both adrenal glands, since they were shown to be a source of estrogens in the postmenopausal woman. The clinical results of Huggins were confirmed by the work of others.[3,4]

Animal investigations by various groups[5-8] indicated that estrogens are

* From the Cancer Research Institute, the Department of Surgery, and the Metabolic Unit, University of California School of Medicine, San Francisco. Part of this work was carried out under Grant C-2085 from the National Cancer Institute, U. S. Public Health Service, and the American Cancer Society, Inc., Institutional Grant, and Grant No. A-288 from the National Institute of Arthritis and Metabolic Diseases. We are indebted to Merck and Co. for cortisone and hydrocortisone, to the Upjohn Company for hydrocortisone, and to the Armour Laboratories and the Wilson Laboratories for supplies of corticotropin.

inactivated in the liver; however, a significant amount of active hormone may pass into the systemic circulation.[9]

It was therefore thought that considerable elimination of estrogens might be accomplished through their inactivation by the liver, obviating the need for the removal of both adrenal glands in humans with metastatic carcinoma.

SURGICAL PROCEDURES

Four patients with advanced metastatic carcinoma of the breast were selected for study. These had been previously treated with mastectomy, irradiation or hormone therapy which had failed to arrest the metastases.

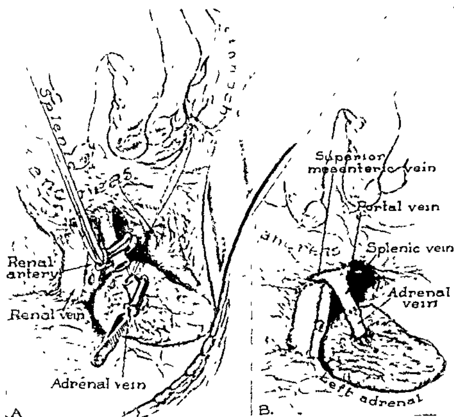


Fig 1 Technique of anastomosing the left adrenal vein to the splenic vein

The surgical procedure chosen was done in two stages: (1) bilateral oophorectomy and right adrenalectomy, (2) three to five weeks later, an end-to-end anastomosis of the left adrenal or renal vein with the splenic vein was made. This procedure was selected in preference to the technically simpler method of direct adrenal implantation into the spleen or mesentery because in previous animal experimentation[10] such transplanted tissue had not survived. Figure 1 illustrates the operative technique, which is outlined in more detail in a previous publication.[4]

The preoperative management of the patient undergoing bilateral oophorectomy and right adrenalectomy was the same as for bilateral adrenalectomy,[4, 11] as there is always the possibility of an atrophic adrenal gland on the left. Postoperative hormonal substitution therapy usually need be con-

tinued for only one or two days. During the second stage, cortisone or hydrocortisone was given before, during, and after the operation.[12] In addition, corticotropin gel in doses of 80 units was given intramuscularly the morning of the operation, and in decreasing dosage daily for approximately 10 days.

CLINICAL RESULTS

The clinical results of diversion of the left adrenal venous blood into the portal circulation of four cases studied are summarized in Table 1. None of the patients required adrenal replacement therapy, demonstrating that the

Table 1. Clinical Results in Patients Following Left Venous Adrenal-Splenic Shunt

PATIENT	AGE	DATE OF SHUNT	HISTOLOGY OF MAMMARY CARCINOMA	LOCATION OF METASTASES	RESULTS	SURVIVAL
I C.	33	March '53	Adenocarcinoma	Soft Tissues	Regression	Still living (Oct. '54)
J P.	54	April '53	Infiltrating	Generalized osteolytic, pulmonary and soft tissues	Progression	Expired (Jan. '54)
E.S.	56	Feb. '51	Infiltrating	Generalized: osteolytic, pulmonary, soft tissues	Progression	Expired (July '54)
L S.	28	March '54	Adenocarcinoma	Soft tissues	Regression	Still living (Oct. '54)

adrenal corticoids were not significantly inactivated by the liver. Only in two of the patients was regression of metastatic lesions noted. The clinical observations were substantiated by determining the levels of urinary 17-hydroxycorticoids and estrogens.

MEASUREMENTS OF URINARY STEROID EXCRETION

Twenty four hour urinary 17-hydroxycorticoids were estimated by a modification of the method of Reddy et al.,[12] and 17-ketosteroids were determined by a modification of the method of Callow et al.[13] In addition to baseline determinations, maximal adrenal secretory capacity was ascertained by the use of the 8 hour intravenous ACTH test.[15]

The changes in 17-hydroxycorticoid excretion of the four patients are shown in Tables 2, 3, 4 and 5 After removal of both ovaries and the right adrenal gland, the control and stimulation values of 17-hydroxycorticoids

are invariably greater than one-half of the preoperative level. This may be due in part to a compensatory functional hypertrophy of the remaining gland. Since 17-ketosteroid excretion does not undergo similar changes a decrease in liver function may account for part of the unexpectedly high 17-hydroxycorticoid excretion. Normally a variable fraction of 17-ketosteroids is formed in the liver by the breakdown of 17-hydroxycorticoids.

*Table 2. Effect of Transhepatic Shunt of the Left Adrenal on Urinary Steroid Excretion**

	17-HYDROXYCORTICOIDS COMPOUND F MG./24 HRS		17-KETOSTEROIDS DEHYDROEPIANDROSTERONE MG./24 HRS.	
TIME	CONTROL	IV ACTH TEST†	CONTROL	IV ACTH TEST†
Before surgery	12	40	10	14
8 days after removal of both ovaries and rt adrenal	8	28	3	5 5
108 days after anastomosing the left adrenal to the splenic vein	7 6	19 5	3 1	6 6
9 months after anastomosis	6 2	21 8	6 1	7 6
17 months after anastomosis	6 2	12 9(?)	4 3	8 2
19 months after anastomosis	5 4	21 0	2 7	11 9

* Patient I C, fem, 35 yrs, U C No 209723

† IV ACTH Test 25 units of ACTH in 1000 cc 5% D/W over an 8 hour period for 2 days

*Table 3 Effect of Transhepatic Shunt of the Left Adrenal on Urinary Steroid Excretion**

	17-HYDROXYCORTICOIDS COMPOUND F MG./24 HRS.		17-KETOSTEROIDS DEHYDROEPIANDROSTERONE MG./24 HRS	
TIME	CONTROL	IV ACTH TEST†	CONTROL	IV ACTH TEST†
Before surgery	4 8	20 4	7 5	12 7
27 days after removal of both ovaries and right adrenal	7 0	23 0	5 5	12.1
81 days after anastomosing the left renal to the renal vein	5 5	19 3	1.8	5.7

* Patient E.S, fem, 56 yrs, U.C No 205257.

† IV ACTH Test: 40 USP units of ACTH in 1000 cc. of 5% D/W over an 8 hour period for 2 days.

Following the venous anastomosis control and stimulation values for 17-hydroxycorticoids do not differ significantly from preoperative values; in patient I.C. (Table 2) there was a gradual decrease in control values, but there were no changes upon stimulation. The output of 17-ketosteroids appears low in control tests, but on stimulation there is a normal response which increased in magnitude during a 19 month period after the anastomosis consistent with changes in 17-hydroxycorticoids. The gradual rise in 17-ketosteroid output on stimulation may be partly explained by improvement in liver function. Conversely, a decrease in 17-ketosteroids in patient E.S. (Table 4) and patient J.P. (Table 5) might be based upon a rapid increase in liver metastases, found to be most extensive at autopsy. The

*Table 4. Effect of Transhepatic Shunt of the Left Adrenal on Urinary Steroid Excretion**

	17-HYDROXYCORTICOIDS COMPOUND F MG./24 HRS.		17-KETOSTEROIDS DEHYDROEPIANDROSTERONE MG./24 HRS.	
TIME	CONTROL	IV ACTH TEST†	CONTROL	IV ACTH TEST†
Before surgery	1.5	20 0		
32 days after removal of both ovaries and right adrenal	9.7	32 0	5 8	12.7
5 months after anastomosing the left renal-splenic vein	6.5	30.0	6.2	11.0
7 months after anastomosis	6.5	25 8	6 0	10 0

* Patient. L.S., fem., 28 yrs., U C. No. 210752.
† IV ACTH Test: 40 USP units of ACTH in 1000 cc 5% D/W over an 8 hour period for 2 days.

*Table 5. Effect of Transhepatic Shunt of the Left Adrenal on Urinary Steroid Excretion**

	17-HYDROXYCORTICOIDS COMPOUND F MG /24 HRS.		17-KETOSTEROIDS DEHYDROEPIANDROSTERONE MG./24 HRS.	
TIME	CONTROL	ACTH TEST†	CONTROL	ACTH TEST†
Before surgery	5	26	7	11
6 days after removal of both ovaries and right adrenal	16	40	3	.
30 days after anastomosing the left adrenal to the splenic vein	14	23.4	1.3	3.8

* Patient: J P., fem., 53 yrs , U C No. 199810
† ACTH Test. 160 USP units of Wilson's Gel Intramuscular (1 injection).

over-all results appear to demonstrate that enough active hydrocortisone-like hormones were secreted to maintain these patients without substitution therapy, and that the shunted adrenal gland remained capable of responding to exogenous corticotropin.

CHANGES IN URINARY ESTROGEN TITERS

Pilot studies were undertaken in an attempt to measure urinary estrogen secretion biologically. We are greatly indebted to Dr. Carl Heller and his associates in Portland, Oregon, for carrying out these determinations by their own method.

Figure 2 summarizes the results on three subjects. Control values, representing the 24 hour urinary output of biologically active estrogens without stimulation of the shunted adrenal gland, are far below the lower limits of

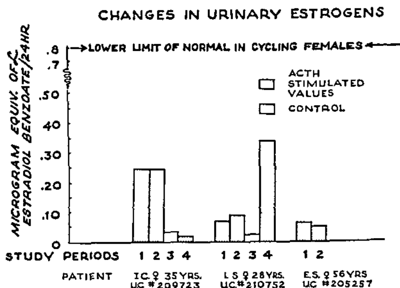


Fig 2 The study period numbers along the base of the graph have the following meaning: *1*, one adrenal in situ, *2*, one adrenal shunted; *3*, control six to nineteen months after shunt, *4*, ACTH test six to nineteen months after shunt

normal premenopausal women (study period 3). This finding is in keeping with the absence of demonstrable effect in the vaginal smears. On corticotropin stimulation there was no significant difference in estrogen output before and soon after the adrenal shunt (study periods 1 and 2). However, while there is a definite decrease in estrogen output in patient I.C. (study period 4, nineteen months after anastomosis), there is a rise in patient L.S. (study period 4, seven months after anastomosis). It is of interest that the former showed marked regression of the carcinoma, while there was a spread of the metastatic process in the latter.

While these pilot studies are too incomplete to allow for a definite conclusion, it would appear that shortly after this procedure there is no significant reduction in the already small output of estrogens from the stimulated adrenal gland. It is obvious that a more refined technique in a greater number of patients must be used to settle the matter of the inactivation of estrogens by the liver under the experimental conditions employed.

DISCUSSION

It has been demonstrated that by transplanting one remaining adrenal into the portal circulation, physiologically sufficient amounts of adrenal corticoids are made available to the organism to maintain life without substitution therapy. Measurements of urinary 17-hydroxycorticoids substantiated the clinical observations. However, this is not a practical therapeutic measure, even though it does eliminate the need for substitution therapy. The procedure has proved useful as a tool for investigation of changes in urinary steroid excretion. In this small group of four patients, two have done well whereas two continued their downhill course and expired. In all four patients the "transplants" survived and proved physiologically adequate.

SUMMARY AND CONCLUSIONS

1. A surgical technique for a study of the role of the liver in the inactivation of estrogens of adrenal cortical origin in the human and its preliminary application in four women with metastatic carcinoma of the breast has been presented.

2. In these patients there was no evidence of adrenal insufficiency up to nineteen months after diverting the effluent left adrenal blood into the portal circulation following bilateral oophorectomy and right adrenalectomy.

3. Urinary 17-hydroxycorticoids were found to be the same after the venous anastomosis as they had been before operation upon corticotropin stimulation. Urinary 17-ketosteroids, low initially, approached normal values gradually following the procedure.

4. Urinary estrogens determined biologically failed to show a fall following the procedure in two patients under corticotropin stimulation. Similar comparisons in the unstimulated stage must await application of more sensitive methods of assay.

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VITAL STAINING OF LYMPHATICS DURING SURGERY*

LAWRENCE H. STRUG, WILLIAM LEON, AND ISIDORE COHN, JR.

A rapid, simple method for determining the extent of spread of carcinoma at the time of surgery would be a most valuable addition to surgical therapy. Neither the palpating finger nor the unaided eye can determine this accurately. Multiple frozen sections are neither practical nor desirable. A colored dye which would differentially outline normal and malignant tissue would be of great aid to the surgeon at the operating table

Direct Sky Blue is believed to be such a dye. The validity of certain concepts has been studied [2, 3] Because of its rapid uptake by the lymphatic system, the limits of malignant disease can be detected within fifteen minutes after the dye is injected into the involved area.[3] Lymph nodes which are stained do not contain carcinoma and unstained nodes are involved by cancer cells.[2, 3] The limits of surgery are determined by remaining in the area of stained or uninvolved nodes.

In addition, Direct Sky Blue is being evaluated in dogs, in humans without carcinoma, and in humans with carcinoma, in an attempt to determine the proper limits of certain operations. Our early efforts have been directed towards further study of carcinoma of the lung and stomach.

TECHNIQUES

A mixture of 5 cc of Direct Sky Blue and 0 2 cc. of hyaluronidase is injected through a fine gauge needle (No 26) into the muscularis and submucosa of the stomach along the greater and/or lesser curvatures and along the anterior and/or posterior surfaces In the lung, injections were of two different types. One was multiple subpleural injections on all surfaces of the lobe whose lymphatics were to be visualized The second was in the bronchial musculature Following the injection, the injected area is not manipulated for 15 minutes to permit adequate uptake of the dye by the lymphatics. This time can be utilized for further exploration of the abdomen or chest. The lung should be inflated to aid spread of the dye. Some lymphatics leading from the points of injection to adjacent nodes will be outlined with dye, and uninvolved nodes along the expected drainage pathway of the lesion will be stained The operation can then be carried out through the area of stained nodes insuring adequate removal of all detectably involved nodes.

Precautions. No human or animal toxicity has been observed from the amount of dye used in these studies. Rapid intravenous injection of 5 cc. of

* From the Department of Surgery, School of Medicine, Louisiana State University, New Orleans, Louisiana. This study was aided by a grant from Wyeth Laboratories.

the dye was without effect in the dog, except for the prolonged bluish discoloration of the skin and mucous membranes that followed. If an inadvertent intravenous injection should occur in a patient, the harmless temporary skin discoloration should be explained to the patient.

The anesthetist should know of this possibility to prevent his misinterpreting the color as cyanosis.

Dye injection in a patient whose lesion is found to be inoperable may also produce discoloration of the skin,[4] although we have not noted this complication.

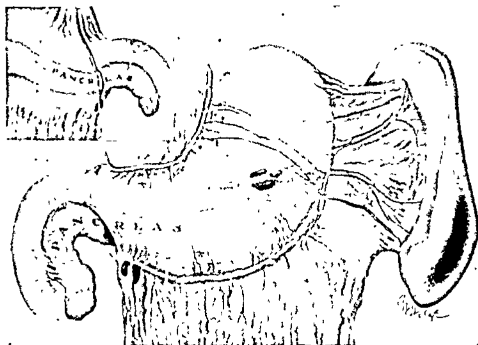


Fig. 1 Composite illustration of the pathways following injection of both curvatures of the dog's stomach. Stars indicate points of injection. Insert shows posterior view of stomach and pancreas to demonstrate pathway across surface of pancreas. See text for details of various dye pathways.

RESULTS

The lymphatic drainage of the stomach has been studied in dogs to evaluate the usefulness of the dye and to outline normal drainage of the stomach. The following areas have been injected: (1) both curvatures on both anterior and posterior surfaces of the stomach, (2) both curvatures on the anterior surface only; (3) one curvature only; (4) certain anatomic portions of the stomach, such as the pyloric area, or the cardia.

While there is some overlap of dye uptake from the various areas, there is enough regional distribution when the dye is injected selectively so that it appears worthwhile for use in outlining lesions of various parts of the stomach.

Six major pathways were observed following injection of the dog's stomach (Fig. 1).

1. Parallel to the two curvatures. These lymphatics followed the vessels

that parallel the curvatures. There was surprisingly little drainage into the omentum.

2. Accompanying the splenic vessels and the vasa brevia and rapidly entering the hilum of the spleen.

3. Into the lymphatics surrounding the duodenum.

4. Across the posterior surface of the stomach and the posterior surface of the body of the pancreas.

5. Directly to the mass of nodes at the base of the dog's mesentery and then intense staining of all the nodes in the mesentery.



Fig. 2 Drawing from a patient with a duodenal ulcer, showing pathways along curvatures and across body of stomach Nodes along the curvatures are demonstrated, in contrast to their absence in the dog

6. Across the body of the stomach joining the greater and lesser curvatures

The dye has been used in humans with benign and malignant gastric or duodenal lesions. In general the lymphatic outlines obtained in patients without carcinoma have been much like those obtained in dogs. It has not been possible to dissect the pancreas so completely to determine if the pathways across the pancreas follow those in the dog. However, in several patients with esophageal carcinoma, where it has been necessary to mobilize the stomach for an intrathoracic anastomosis, dye has been observed crossing the pancreas in a fashion similar to that observed in dogs. Staining of the mesenteric nodes in the dog has not been correlated with anything in

man, since such a collection of nodes does not exist in man, and we have not felt justified in dissecting the entire abdominal lymphatics in a benign lesion. Nodes along the two curvatures have been well demonstrated in several benign cases, and lymphatics leading to the spleen have been well outlined (Fig. 2).

In a few cases of gastric carcinoma this dye has also been used with success. It has been possible to inject near the lesion and note that the nearest nodes do not take the stain, while others further away have been well

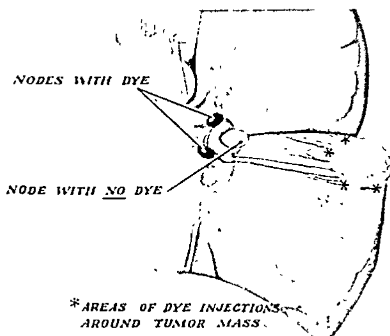


Fig. 3. Illustration of dye injection around carcinoma of the lung, demonstrating lymphatic pathways to nodes at hilum and single node filled with carcinoma which failed to take the dye.

stained. Histologically the stained nodes have not contained carcinoma, while those that were not stained have been either partially or completely replaced by carcinoma. Partial staining may be correlated with partial replacement by carcinoma. This phenomenon was also observed in the tracheobronchial lymph nodes.

When a lobe of the lung was injected subpleurally, lymph nodes in the mediastinum were stained, and their location depended upon which lobe was selected (Fig. 3).

The drainage from the upper lobes is to the tracheobronchial, paratracheal and carinal lymph nodes. The middle lobe lymphatics drain into the depths of the horizontal fissure and then into the anterior tracheobronchial lymph nodes. When the posterior surface of the lower lobe was injected on the right side, lymphatics along the mediastinum were visualized inferior to the diaphragm. More important was the staining of lymph nodes in the opposite mediastinum in dogs. This included the paratracheal and tracheobronchial lymph nodes. The importance of this observation is evident in the light of the more radical attempts to remove all mediastinal lymph

nodes. The method proposed would limit the amount of dissection that is necessary in the mediastinum.

We believe that one accidental observation is of considerable significance. After the stomach is injected, the dye goes to the nodes in the base of the mesentery, to the cisterna chyli, and then traverses the thoracic duct The entire length of the thoracic duct is intensely stained. Thus immediate visualization of the thoracic duct is possible and injury to this delicate structure can be avoided. In addition, the same staining of the thoracic duct can be obtained by injection of the esophagus, so that this may have immediate clinical application for intrathoracic procedures.[1] We are now studying this phenomenon clinically.

CONCLUSIONS

1. A dye, Direct Sky Blue, may be used for vital staining of abdominal or thoracic lymphatics at surgery.
2. Lymph nodes that are not invaded by carcinoma will take the dye while those invaded partially or completely by carcinoma will not become discolored by the dye.
3. No toxic manifestations have been noted in either animals or humans.
4. Evaluation of the lymphatics of the stomach and lung in the dog has suggested that dissemination of carcinoma may be more widespread than has been thought previously.
5. The thoracic duct can be stained during either abdominal or thoracic procedures, and the clinical applications of this have been noted.
6. The dye has been used effectively in clinical cases of carcinoma of the stomach and lung, and has indicated the limits of resection that would be feasible in these two areas. Further study of the lymphatics in humans with malignancy is indicated and is under way.

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ANESTHESIOLOGY

INTRODUCTION

HENRY K. BEECHER

Somewhere there is a paradox in the fact that to take out a lung requires more training and experience and learning and judgment than it does to take away consciousness, yet the loss of consciousness has immeasurably greater effect on the individual. *All effective existence ceases with this loss. With* the loss of a lung an individual can lead an essentially normal life. A statement of what the anesthetist does deliberately to the body and mind, if one looks at what is done and gives no attention to how these things are accomplished, presents an awesome spectacle. Let us take a brief look at the physical and mental changes the anesthetist more or less commonly produces: he controls the vital sensations of consciousness, of respiration, of circulation, of neuromuscular function and of metabolism, and all of these things he does under the adverse circumstances of preceding disease and present surgery. (It is curious that it is easier to take away consciousness, with its vast consequences, than it is to take away a lung.)

Spectacular as some accomplishments have been in the field of anesthesia, one must admit that there are difficulties and problems associated with each of these procedures and that death sometimes follows. One can ask, is there any *wonder that the acts the anesthetist just mentioned are not without* serious consequence, not without fatal results? The great wonder is that the things just mentioned can be carried out with the death rate as low as it is. The anesthetist in recent decades has accomplished much, or to put it more accurately, has utilized much discovered by others. The anesthetist's application of these discoveries gives him perhaps some right to take pride in the extraordinary things he can do to the body and to the mind. There are, however, areas where general performance is still far from ideal: Consider here the disproportionately high anesthesia death rate in infants and small children. It is not yet clear whether these difficulties are to be explained by the fairly recently recognized physiologic immaturity of these small citizens or whether the technical difficulties of working with small size is at the heart of the problem. Deaths do occur as a result of anesthesia in all sizes and in all ages and they occur more commonly than some are willing to admit. *But the fact remains that great progress has been made.* While one must admit that a first step in the progress is to face the facts, however unpleasant they may be, one's consideration need not be left at such a gloomy level. The papers presented at the Forum on Fundamental Surgical Problems make it very clear that many problems which still remain unsolved in the field of anesthesia are being attacked vigorously and in a promising way.

In these developments in anesthesia it is of the utmost importance to keep clearly in mind which of the three categories a given advance falls in. There are advances in anesthesia which have made it safer. These are very few. There are advances which have made it less unpleasant for the patient or

more convenient for the surgeon. These are important and numerous but not to be confused with those in the first category just mentioned. And finally, there are a few advances which have made heretofore impossible surgical procedures possible It is essential that advances be understood in these or similar terms.

THE EFFECTS OF NARCOTICS UPON THE RESPIRATORY RESPONSE TO CARBON DIOXIDE IN MAN*

JAMES E. ECKENHOFF, MARTIN HELRICH, AND MURIEL J. D. HEGE

These studies were undertaken as part of a study of respiratory acidosis during anesthesia and operation. A system for recording suitable data in anesthetized or unanesthetized patients has been developed. We have employed this method in 33 normal unanesthetized subjects and 12 patients, 8 of whom were anesthetized. The data reported herein pertain to the effects of opiates on man. They suggest that opiates be omitted from preoperative medication except in unusual circumstances.

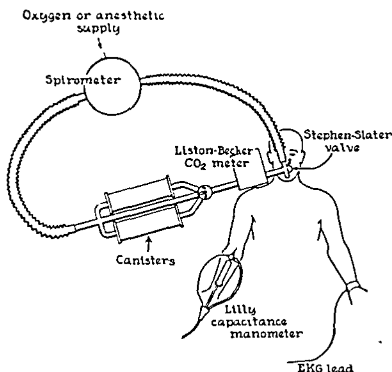


Fig. 1.

METHOD

The method employed is presented diagrammatically in Figure 1. It has been described in detail elsewhere.[1] A closed circle filter system has been devised incorporating a small spirometer, an infra-red carbon dioxide analyzer (Liston-Becker), and carbon dioxide absorption canisters which can be shunted out of the system. Unidirectional flow of respired gases is produced by respiratory valves (Stephen-Slater) placed close to the pa-

* From the Department of Anesthesiology, Hospital of the University of Pennsylvania, and the Harrison Department of Surgical Research, University of Pennsylvania School of Medicine, Philadelphia.

tient's mouth. Continuous respiratory tracings have been obtained for as long as 8 hours. End-expiratory carbon dioxide concentrations are recorded on a two-channel recording oscillograph. Intra-arterial blood pressure records using the Lilly capacitance manometer and electrocardiographic tracings are recorded. General anesthetics can be administered from an anesthesia machine into the spirometer.

The inhalation of varying concentrations of carbon dioxide is a recognized test of the reactivity of the respiratory center.[2,3] If the center is depressed

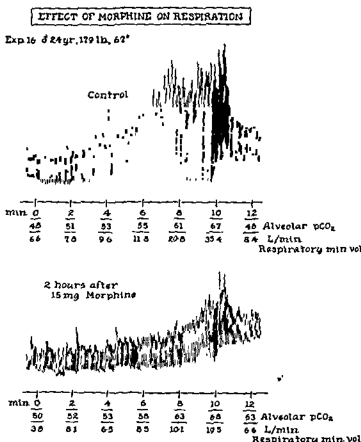


Fig. 2

by drugs, respiratory response to carbon dioxide is reduced. In these experiments, after suitable control periods, the patient's response to endogenously accumulated carbon dioxide (by-passing the soda lime canister) was tested. An opiate, barbiturate, or general anesthetic was then administered and the test repeated at varying intervals

In validating the method, the following facts have been established[1]: (*a*) The carbon dioxide meter incorporated into the system as described measures end-expiratory carbon dioxide concentration and allows for a reasonably accurate estimation of alveolar pCO_2* so long as tidal volume is adequate to clear the respiratory dead space. (*b*) The results obtained with

* In 63 tests, end-expiratory pCO_2 was 1 4 ± 1 3 mm. Hg lower than the pCO_2 in maximal expirations

the method are reproducible in the same subject on the same day or on different days.

The opiates studied include morphine, meperidine, Dilaudid, codeine, methadon, Nisentil,* Dromoran Hydrobromide,* and nalorphine. The effects of these drugs have been compared with those of secobarbital.† All medications have been given intramuscularly and in therapeutic dosage.

RESULTS

The upper tracing in Figure 2 depicts the response of a subject to endogenously accumulated carbon dioxide under control conditions. The lower tracing shows the response in the same individual 2 hours after 15

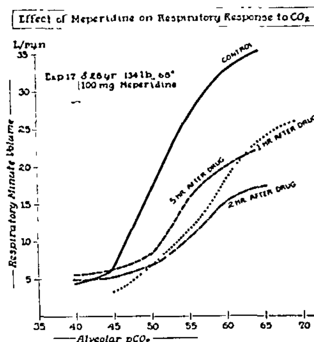


Fig. 3.

mg. morphine sulfate had been injected intramuscularly. The severe respiratory depression is obvious. In 2 of the 3 individuals to whom morphine was given, maximal respiratory depression was still present 4 hours after administration of the drug. In the third, maximal depression was reached in 3 hours.

Figure 3 demonstrates the duration of respiratory depression as measured by response to carbon dioxide accumulation in a subject to whom 100 mg of meperidine had been administered intramuscularly. Significant depression still existed 5 hours after injection of the drug

Table 1 lists the duration of respiratory depression and of other symptoms associated with the administration of opiates in 22 subjects. Pertaining to these later symptoms, the following were interesting: (*a*) a biphasic response with a pleasant, comfortable sensation for an hour or two after injection of the drug followed by general restlessness and unhappiness; (*b*)

* Supplied through the courtesy of Hoffmann-LaRoche Company, Nutley, New Jersey.
† Supplied through the courtesy of Eli Lilly Company, Indianapolis, Indiana.

a prolonged (2 to 24 hours) period of mental depression associated with vertigo, nausea, lassitude and anorexia. The latter was most notable following morphine, Dromoran, and Nisentil.

In these 22 subjects, the average increase in alveolar pCO_2 following administration of opiates was 6 mm. Hg with a range of 2 to 12 mm. Hg.

Table 1.

DRUG	DOSE MG	NO OF SUBJECTS	DURATION OF RESPIRATORY DEPRESSION HOURS	DURATION OF OTHER SYMPTOMS HOURS
Morphine	15	3	1, 1*, 4*	7, 24, 20
Codeine	60	2	2, 2	2, 3½
Meperidine	100-125	3	4, 1, 5*	5, 2, 5
Dilaudid	2-3	3	2*, 1, 5*	5, 3, 3
Dromoran	5 0-7 5	3	2, 2*, 3½*	10, 20, 24
Methadon	7 5-8 0	2	4*, 4*	6, 7
Nisentil	45-60	3	1*, 2, 2	10, 10, 5
Nalorphine	10-15	3	3*, 0, 1	6, 2½, 3

*Indicates depression was present with last measurement

Also of interest is the fact that while 18 of the 22 subjects demonstrated no change in respiratory rate or increased respiratory rate after opiates, in 16 of the 18 there was a reduction in pulmonary ventilation because of a diminished tidal exchange.

Opiates in comparable dosage were administered to patients who had been scheduled for operation or who were experiencing severe and chronic

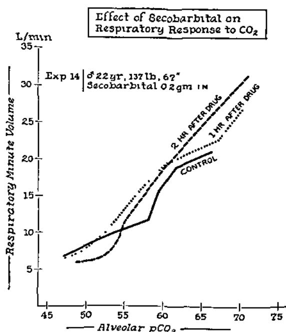


Fig. 4.

pain. No essential difference was noted in the response of these patients as compared with the normal subjects. In several patients with chronic pain, it was noticed that respiratory depression as evidenced by increase in alveolar pCO_2 and diminished respiratory response to endogenously accumulated carbon dioxide would persist in spite of return of pain. Two patients suffered the return of severe pain 3 hours after the administration of methadon or Nisentil, yet demonstrated the same degree of respiratory depression as was evident 2 hours after injection of the drug. Another individual demonstrated marked respiratory depression after meperidine, although he experienced pain all through the study.

In order to compare the respiratory response of patients to whom barbiturates had been administered, 3 subjects were given either 100 or 200 mg. of secobarbital sodium. The data from one subject are presented graphically in Figure 4. The other 2 subjects responded similarly. None of the subjects demonstrated respiratory depression, although 2 of the 3 were sleepy for 7 hours following administration of the drug.

DISCUSSION

The fact that opiates are potent drugs producing widespread and prolonged effects in man has been demonstrated many times. However, there is still a tendency to prescribe these depressants without regard for their potency and prolonged action.

The results of the experiments reported herein emphasize three things: (1) The duration of action of a single dose of opiate may exceed 24 hours, even though pain relief may disappear within a few hours. (2) The respiratory depressant effect may not be readily apparent except by careful observation. Reduced pulmonary ventilation may be present in spite of an apparently adequate respiratory rate. The latter is of interest in view of the commonly accepted practice of using respiratory rate as a guide to the repeated injection of opiates for pain relief. (3) Respiratory depression may persist in spite of return of pain, usually the signal for the administration of more opiate. Diminished pulmonary ventilation producing high carbon dioxide levels and occasionally even low blood oxygen levels may spell danger for the patient in a precarious state. Evidence is increasing that respiratory acidosis may predispose to cardiac irritability[4] and hypotension[5] even in the normal patient.

Opiates have become a time honored part of the preparation of patients for anesthesia and operation. In most clinics, seldom is a patient sent to the operating room unless he has had an injection of an opiate and belladonna drug. The advocacy of this standard procedure has been questioned.[6] Opiates are given primarily to produce sedation preanesthetically; however, their effect upon respiration, circulation, and other body systems may long outlast the duration of the anesthetic. Our data suggest that such sedation could be better produced by barbiturates or other sedatives with less undesirable side effects rather than by opiates. Anesthetic agents and the stress of operation offer sufficient problems in themselves. Are we justified in [illegible] :h produce prolonged respiratory [illegible] 1 dioxide levels, and reduce the [illegible] vasomotor control? We are at present investigating this question.

SUMMARY

A technique for studying the respiration and circulation in the unanesthetized or anesthetized patient is described. A preliminary series of studies in subjects and patients to whom opiates or barbiturates were administered has been presented. These studies re-emphasize the prolonged and widespread actions of opiates and reopen the question of the desirability of administering narcotics prior to anesthesia and operation.

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THE EFFECT OF SURGICAL POSITIONS ON RESPIRATION*

JOHN R. JONES AND JAY JACOBY

The position of a patient on the operating table influences respiration. This has been shown by changes in the vital capacity[1,3,6,7] and by noting the deleterious effects on the circulation and respiration brought about by extreme positions on the operating table[5,8] Alterations of vital capacity, however, do not necessarily indicate changes in the respiration of anesthetized patients, since vital capacity measures the maximum respiratory effort in a conscious cooperating individual.

The tidal volume is the amount of air which is moved in and out of the respiratory tract with each breath Voluntary effort by the patient is not required or desired in its measurement. Determination of tidal volume thus gives a more accurate method of evaluating changes in respiration while under the influence of an anesthetic.

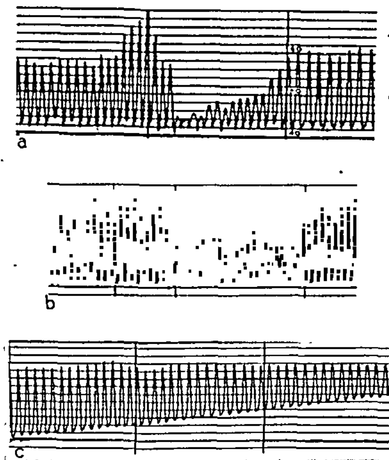
METHOD

The patients in this study were adults of both sexes and various body builds. They had no evidence of respiratory or other serious disease, having

* From the Department of Surgery, Section of Anesthesiology, Ohio State University College of Medicine, Columbus

been brought to the operating room for minor procedures. After anesthesia was established the patients were placed in various positions and recordings of tidal volume were made. A balanced anesthesia was used, consisting of either Pentothal-cyclopropane-curare or Pentothal-nitrous oxide-curare.

The tidal volume, or respiratory exchange, of anesthetized patients was measured by interposing in the anesthesia equipment a basal metabolism machine by means of which continuous tracings could be obtained. Special



rest is elevated, and the return to previous exchange when the rest is lowered. *c*, Shows progressive increase of respiratory excursion as the depth of anesthesia is reduced.

care was taken to maintain the same plane of anesthesia and to avoid respiratory obstruction, so that the tracings would give an accurate indication of the changes in respiration caused by changing the position of the patient. Figure 1 shows typical changes in tidal volume due to (1) obstruction, (2) change in position, and (3) altering the level of anesthesia.

RESULTS

The average tidal volume of anesthetized patients is somewhat below the

SUMMARY

A technique for studying the respiration and circulation in the unanesthetized or anesthetized patient is described. A preliminary series of studies in subjects and patients to whom opiates or barbiturates were administered has been presented. These studies re-emphasize the prolonged and widespread actions of opiates and reopen the question of the desirability of administering narcotics prior to anesthesia and operation.

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METHOD

The patients in this study were adults of both sexes and various body builds. They had no evidence of respiratory or other serious disease, having

* From the Department of Surgery, Section of Anesthesiology, Ohio State University College of Medicine, Columbus

space averages 150 cc. Effective alveolar exchange equals tidal volume (500 cc.) minus dead space (150 cc.), or 350 cc. per breath, in the conscious person (Fig. 3A).

Under anesthesia tidal volume is frequently reduced to 400 cc. The anesthetic mask adds at least 50 cc. to the dead space, making the total dead space about 200 cc., and reducing the alveolar ventilation to 200 cc. per

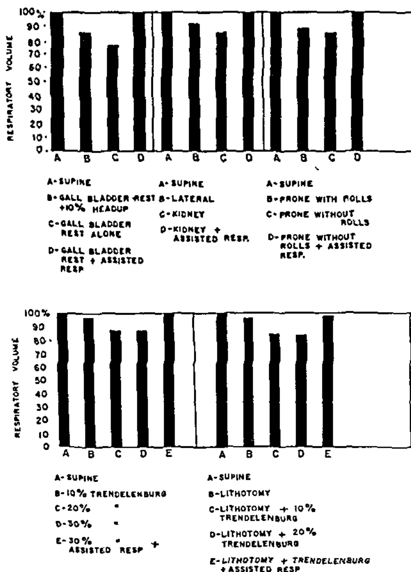


Fig. 2. Effects of surgical positions on respiratory volume

breath (Fig. 3B). If tidal volume is further reduced by 25 per cent (100 cc.), owing to position, the entire loss is sustained by the alveolar exchange since the dead space remains constant. Alveolar exchange in this example is reduced to 100 cc. (Fig. 3C), compared to the 350 cc. normal. This promotes carbon dioxide retention and acidosis. When the patient is breathing a high oxygen mixture, anoxia is not likely to occur; if the gas mixture is low in oxygen, however, anoxia is superimposed on the acidosis.

The use of an endotracheal tube decreases dead space to about 75 cc.[4] by

500 cc. per breath which is considered normal in conscious people. This is presumably due to the fact that there is a diminished need of the tissue cells for oxygen and diminished rate of metabolism caused by loss of consciousness and decreased reflex irritability. In addition, and more important, the anesthetic agents depress the respiratory center and cause a diminished respiratory effort, while curare and other muscle relaxants cause paralysis of the muscles of respiration.

The important thing to note in this study, however, is the effect of the commonly used positions for operation on the respiratory amplitude. Using the supine position as the standard, the tidal volume was moderately reduced (14 per cent) by the kidney, prone, and steep Trendelenburg positions, and greatly reduced (25 per cent) by elevation of the gallbladder rest (see Table 1). Emphasis should be placed on the fact that the effect of

Table 1. Effects of Position on Tidal Volume

POSITION	DECREASE IN TIDAL VOLUME
10° Trendelenburg	3%
Lithotomy	3%
Lateral	8%
Prone with support	11%
20° Trendelenburg	12%
30° Trendelenburg	12%
Prone without support	14%
Kidney	14%
Gallbladder rest + 10° head up	14%
Lithotomy + 10° Trendelenburg	14%
Lithotomy + 20° Trendelenburg	15%
Gallbladder rest	24%

packs and retractors has not been included in this study, pressure on the diaphragm might well cause an even greater decrease in tidal volume

In general, the body build and the depth of anesthesia had little effect on the percentage of change in the tidal volume from one position to another, although with subjects in a deeper plane of anesthesia the initial tidal volume was lower. There was a suggestion that the more obese individuals tended to have a slightly greater effect from the change in position. The type of anesthetic used, the sex and the age of the individual had no effect.

DISCUSSION

sis is counteracted by the use of assisted respiration which increases tidal volume. When patients are placed in positions which reduce their tidal volume, there is a greater need for assisted respiration (see Fig. 2).

In a consideration of respiratory exchange, the effect of dead space must be emphasized. The gases in the mouth, pharynx, trachea and bronchi do not participate in alveolar ventilation and represent the dead space. The dead

harmful to the patient unless it is compensated for by assisted respiration. The use of positioning to facilitate surgical procedures on healthy patients is not contraindicated by the effect of position on respiration inasmuch as it is possible to increase the tidal volume by means of assisted respirations.

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THE EFFECT OF UNILATERAL REBREATHING OF LOW OXYGEN GAS MIXTURES UPON THE PULMONARY BLOOD FLOW IN MAN*

W. S. BLAKEMORE, E. CARLENS, AND S. BJÖRKMAN

Although considerable discussion has followed the publication of von Euler and Liljestrand in 1946[1] when they reported a rise in pulmonary artery pressure in the cat during hypoxia, it remains unproven that perfusion of the human lung locally is controlled to some degree by alveolar or blood gas tensions. If such a mechanism exists it is of importance to establish it as a basis of further understanding of the principles which regulate pulmonary blood flow. In pathologic conditions other mechanisms are known to affect the redistribution of blood flow. These mechanisms undoubtedly include physical and mechanical forces and perhaps further regulation is affected by means of neurogenic or chemical stimuli in addition to factors yet unknown. Nisell[2] has made an extensive study of the changes in blood flow through the isolated lungs of cats and rabbits and found that low oxygen or high carbon dioxide concentrations in the inspired gas increased the pulmonary vascular resistance, with high oxygen tension in the blood, he concludes that the pulmonary venules dilated while with an increase in the carbon dioxide tension in the blood, the pulmonary venules constricted. The pulmonary arterioles reacted in the opposite manner. The exact site of the vascular constriction and dilatation may be challenged by other investigators, but many are in agreement with these findings of the changes in pulmonary vascular resistance and pulmonary blood flow.

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eliminating the space of the mouth and pharynx. This diminishes the harmful effect of reduced tidal volume on the alveolar exchange. Continuing the example above, with the tidal volume under anesthesia of 400 cc. and a dead space of 75 cc. the alveolar exchange is 325 cc. (Fig. 3*D*). After a position change which decreases tidal volume to 300 cc., the alveolar exchange becomes 225 cc. (Fig. 3*E*). This is evidently much more satisfactory than the alveolar exchange of 100 cc. obtained with a mask.

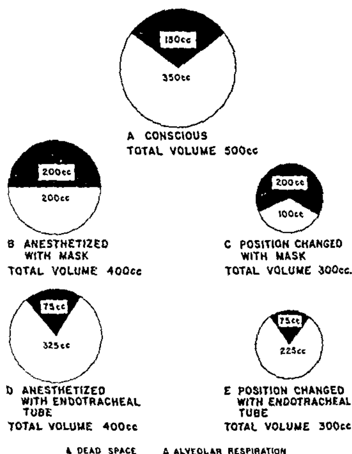


Fig 3. The effect of dead space on alveolar ventilation in patients with reduced respiratory excursion.

Although the effect of position on the cardiovascular system was not emphasized in this study, frequent observation of the pulse and blood pressure showed no important changes. Significant alterations in the circulatory system might occur if poor risk patients were kept in extreme positions for prolonged periods.

SUMMARY

The effects of various surgical positions on the tidal volume of normal anesthetized patients are presented. Various positions cause progressive decreases in tidal volume, the greatest drop (25 per cent) being caused by elevation of the gallbladder rest. Dead space becomes more important as tidal volume is reduced. It is pointed out that diminished tidal volume is

Table 1. The Percentage of Total Cardiac Output through the Individual Lung During Unilateral Hypoxia in the Supine and the Lateral Decubitus Position

PATIENT AGE SEX LESION	CARDIAC OUTPUT LITER/MIN.	TIME OF UNILATERAL HYPOXIA, MINUTES	% OF CARDIAC OUTPUT THROUGH EACH LUNG RT	LT.	REMARKS
SERIES I					SUPINE AND LATERAL DECUBITUS POSITION
B E.	—	0	49	51	Control—supine
38 M.	12 25*	8	87	13	Low O_2 to lt. lung—supine
Tbc. Rt. U.L.	8 35*	25	74	26	Low O_2 to lt. lung—lt. lat. decubitus
G M H.	8 53*	0	53	47	Control—supine
	8 54†	0	53	47	
27 F.	9 23*	9	80	20	Low O_2 to lt. lung—supine
Tbc. Rt. U.L.	9 30*	30	86	14	Low O_2 to lt. lung—rt. lat. decubitus
R.A.	12 38*	0	58	42	*Control—supine*
	10 28†	0	58	42	
29 M.	22 85*	10	53	47	Low O_2 to rt. lung—supine
	19 58†	12	50	50	
Tbc. Lt. U.L.	14 55†	25	66	34	Low O_2 to rt. lung—rt. lat. decubitus
S E.	18 58†	0	66	34	Control—supine
38 M	21 21†	12	61	39	Low O_2 to rt. lung—supine
Tbc. Lt.	13 89†	33	59	41	Low O_2 to rt. lung—lt. lat. decubitus
A D.	7 58*	0	75	25	Control—supine
	9.12†	0	75	25	
	6 86*	10	65	34	Low O_2 to lt. lung—supine
19 M.	7 76†	12	69	31	
Pericardial	8 36*	28	49	51	Low O_2 to lt. lung—lt. lat. decubitus
Diverticulum	7 39†	30	57	43	
SERIES II					ALL SUPINE POSITION
B.S	22 00*	0	53	47	Control
	16 00†	0	53	47	Control
30 M.	14 70*	11	69	31	Low O_2 to lt. lung
Thymoma	16 00*	28	63	37	Low O_2 to lt. lung
	14 80*	36	60	40	Low O_2 to lt. lung
	13 60†	37 5	58	42	
K.N.	7 60*	0	44	56	Control
	8 30†	0	44	56	Control
19 M.	7 57*	10	76	24	Low O_2 to lt. lung
	7 77†	13	79	21	Low O_2 to lt. lung
Calcified	9 95*	28	62	38	Low O_2 to lt. lung
Pleuritis	9 56†	30	68	32	Low O_2 to lt. lung
Rt. L.L.	10 50*	37	54	46	Low O_2 to lt. lung
S B.	14.30*	0	49	51	Control
	16 55†	0	49	51	Control
38 M.	9.19*	7	71	29	Low O_2 to lt. lung
	10 20†	8 5	71	29	Low O_2 to lt lung
Tbc Rt. U.L.	16.50*	25	55	45	Low O_2 to lt. lung
	10.40†	28	59	41	Low O_2 to lt. lung
	13.50*	32 5	72	28	Low O_2 to lt. lung
	7 86†	34	79	21	Low O_2 to lt. lung
A H.	—	0	50	50	Control
34 M.	13.00*	10	62	38	Low O_2 to lt lung
	13.10†	[illegible]	[illegible]	[illegible]	[illegible]

The present experiments were initiated to determine the effect of unilateral rebreathing of gas containing low concentration of oxygen upon the distribution of pulmonary blood flow in man and to determine the influence of lateral decubitus position upon the pulmonary blood flow through each lung.

Early work by Bjorkman[3] and a number of recent investigators[4, 5] has shown that ventilation and oxygen uptake were increased in the under lung in the lateral decubitus position.

METHODS AND MATERIAL

Nine patients with small intrathoracic lesions were used in these examinations (Table 1). Blood samples were obtained from a catheter in the pulmonary artery and a needle in the brachial artery Bronchospirometry was done using a Carlens double lumen tube after the usual premedication and topical anesthesia [6] Control studies were made with the patient lying in the supine position while both lungs inspired a mixture of gas consisting of air enriched with oxygen. The cardiac output was determined using Fick's principle and the blood samples were analyzed for oxygen and carbon dioxide by Hilty's modification of the Riley, Proemmel and Franke tension method[7] and the method of Van Slyke-Neill. Additional correction of the percentage of oxygen saturation in the blood was made for changes in hemoglobin* and for the oxygen dissolved in the plasma.[8] The content of oxygen in the blood samples was determined from the tension values with the same corrections and with correction for the carbon dioxide tension using the curves of Henderson.[9] Oxygen consumption was measured from

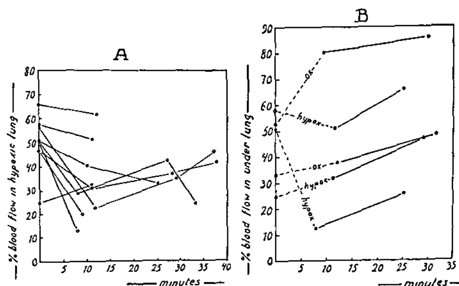


Fig 1. The distribution of the pulmonary blood flow during the control period, and during rebreathing of alveolar gas by one l
mixtures of a high oxygen concentration A,
for the hypoxic lung B, Patients were lying fi
decubitus position, all values were for the under lung

* We are indebted for the determination of total hemoglobin to Dr. Bo Norberg, Director of the Clinical Laboratory, Sabbatsberg Hospital.

after five minutes of rebreathing, this gas mixture proved to be near equilibrium. In most instances no further change was found in the equilibrium base line of the bronchospirometric tracing. After 10 minutes all of these patients showed a decrease in the percentage of total cardiac output flowing through the lung rebreathing the artificial alveolar gas mixture. Additional determinations of the relative flow through each lung were made at 25 and 35 minutes. After 25 minutes three of the patients showed a secondary increase in the relative blood flow through the hypoxic lung which left the blood flow still less than during the control period. The arterial oxygen tension in the blood from the pulmonary artery did not show any consistent change while the carbon dioxide tension of the pulmonary artery showed only a small, and probably insignificant, rise. No significant change in pulmonary artery pressure was noted in these four patients, nor was there any consistent change in the cardiac output in the two series of patients.

From calculations of the percentage of total ventilation contributed by each lung during the control period and during the period of unilateral rebreathing of alveolar gas mixtures there was no significant change which could be construed to be due to constriction of the bronchial tree secondary to the difference in oxygen or carbon dioxide tension in the respired gases.

COMMENT

Two separate determinations of cardiac output were made within several minutes of each other at 10, 25 and 35 minutes. The oxygen and carbon dioxide content of the first blood samples were analyzed by the tension method, and the second blood samples by the Van Slyke method. The variations in the determinations of cardiac output by these methods are shown in Table 1. These variations are usually magnified in determining the percentage of total cardiac output through each lung. While the A-V difference for oxygen by the tension and content method may be approximately equal, an error in the estimation of the oxygen content of the sample by the methods is reflected by a greater error when estimating the relative blood flow through each lung. Although precise measurements of the changes in the relative blood flow cannot be made, the data demonstrate that these changes in blood flow through each lung are of sufficient magnitude to show a consistent trend (Fig 1), regardless of the errors in the method. The one patient who did not show a decrease in the blood flow through the hypoxic lung was a patient who had abnormal bronchospirometric values during the control period. In such a patient other controlling factors are undoubtedly present which may influence pulmonary blood flow even more than changes in oxygen and carbon dioxide tension in the blood or alveolar gas. There could not exist great disparity between ventilation and perfusion if blood flow was controlled only by alveolar gas tensions. Additional evidence that another controlling mechanism exists is presented by the increase in the relative blood flow through the under lung when the patients were turned [illegible] of the main factors for this increase [illegible]ler lung is probably that of gravity. [illegible] the diaphragm of the under side and changes in the "respiratory pump" mechanism is difficult to evaluate without more complete information of the mechanics of respiration of the individual lung and the change in lung volumes in this position. The increase in the relative blood flow in the under lung cannot in all cases be due to

the bronchospirometric tracings. Gas samples from the spirometer were analyzed by the method of Haldane,[8] and the pulmonary artery pressure was measured in the patients of series II by a saline manometer.

After a control bronchospirometric study one of the spirometers was filled with a stock alveolar gas mixture which consisted of 83 per cent nitrogen, 5 per cent carbon dioxide and 12 per cent oxygen in the patients in series I, or 88.5 per cent nitrogen, 4.8 per cent carbon dioxide and 6.7 per cent oxygen in the patients in series II.

Potassium hydroxide was used for the absorption of carbon dioxide from the circuit of the contralateral side while the patients rebreathed on this side a gas mixture which consisted of approximately 80 per cent oxygen Duplicate blood samples were drawn simultaneously from the pulmonary and brachial arteries after 10, 25 and 35 minutes of rebreathing. Gas samples were taken from the spirometers at the end of each experiment. The patients in series I were turned from the supine to the right or left lateral decubitus position after approximately 10 minutes of unilateral hypoxia. The blood flow through the oxygen breathing lung during the period of rebreathing low oxygen mixtures on the other side was assumed to reach 100 per cent saturation and corrections were made for the dissolved oxygen in the plasma at the oxygen tension of the blood sample obtained from the brachial artery No correction was made for the shunting of blood while both lungs breathed high oxygen during the control period As estimated from the clinical findings, the more abnormal lung was always maintained on a high oxygen mixture. If the blood flowing through this lung did not reach full saturation it would appear in the calculations as greater flow through the hypoxic lung.

The calculations were made as follows. Total flow was determined by the measurement of the cardiac output The flow through the hyperoxic lung was determined by the oxygen absorption from this lung, assuming that the blood entered the lung at the same saturation as the pulmonary artery blood and left the lung 100 per cent saturated The flow through the hypoxic lung was obtained by assuming that the blood passed through the lung without *changing oxygen saturation and mixed with the blood from the hyperoxic* lung in the left side of the heart. The amount contributed by each lung could be calculated from the determinations noted above for the total flow, the flow through the hyperoxic lung and measurement of the oxygen content in the blood of a systemic artery.

RESULTS

Of the patients in series I, four of the five showed a decrease in the percentage of total cardiac output passing through the hypoxic lung after 10 minutes. The one patient who did not have such a decrease was one of the two who had markedly abnormal bronchospirometric values during the control period These five patients were then turned into the lateral decubitus position and after 15 additional minutes all were found to have an increase in the percentage of total cardiac output flowing through the under lung (Fig. 1*B*). In two of these patients the under lung was rebreathing from the spirometer with high oxygen concentration and in the other three patients the under lung was rebreathing the artificial alveolar gas mixture.

In the patients from series II after a similar control period one lung was connected to a spirometer containing the artificial alveolar gas mixture and

after five minutes of rebreathing, this gas mixture proved to be near equilibrium. In most instances no further change was found in the equilibrium base line of the bronchospirometric tracing. After 10 minutes all of these patients showed a decrease in the percentage of total cardiac output flowing through the lung rebreathing the artificial alveolar gas mixture. Additional determinations of the relative flow through each lung were made at 25 and 35 minutes. After 25 minutes three of the patients showed a secondary increase in the relative blood flow through the hypoxic lung which left the blood flow still less than during the control period. The arterial oxygen tension in the blood from the pulmonary artery did not show any consistent change while the carbon dioxide tension of the pulmonary artery showed only a small, and probably insignificant, rise. No significant change in pulmonary artery pressure was noted in these four patients, nor was there any consistent change in the cardiac output in the two series of patients.

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increased oxygen tension secondary to hyperventilation because in these experiments patients who showed a marked increase in relative blood flow included those who had been turned with the under lung rebreathing the alveolar gas mixtures.

Still other observations of the pulmonary blood flow during atelectasis or in the open thorax during pneumonectomy[10] show that the gas tension is not the only regulating factor. During these special circumstances the change in blood flow may occur more slowly.

There was only a slight change in carbon dioxide tension in the pulmonary artery blood entering the lung during the test period but the blood leaving the alveolus had a higher carbon dioxide tension during the test period than in the control period. No conclusions as to the effect of carbon dioxide upon the distribution of pulmonary blood flow in man can be made.

SUMMARY

Two of the factors, gas tension and position, which control the distribution of pulmonary blood in man were studied. Eight of nine patients showed a redistribution of the pulmonary blood flow with a decrease in flow through the hypoxic lung with unilateral rebreathing of alveolar gas mixtures.

Five of the patients were studied in the lateral decubitus position as well as in the supine position. All showed an increase in blood flow through the under lung whether rebreathing low or high oxygen gas mixtures in this lung.

It appears that local oxygen or carbon dioxide tension and the body position influence the distribution of pulmonary blood.

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FACTORS AFFECTING CONTRALATERAL VENTILATION DURING UNILATERAL ATELECTASIS*

W. ANDREW DALE AND HERMANN RAHN

Acute pulmonary atelectasis is commonly associated with gross changes in the respiration. With the development of methods of studying the separated lungs of dogs[1] it has been possible to demonstrate the changes that occur in the respiratory pattern of the open lung when the other is made atelectatic. The principles governing these changes appear to be valid for atelectasis involving portions of pulmonary tissue other than an entire lung.

The amount of pulmonary tissue available for gas exchange is reduced by acute atelectasis and it may be assumed that immediate adjustments of ventilation will occur. This adjustment or reaction may be due to various mechanisms acting alone or together. This study was done to evaluate the role of these factors, namely, (1) nervous reflexes, (2) blood chemical changes, and (3) mechanical factors.

METHODS

By means of a bilumen plastic endotracheal catheter (placed during open left thoracotomy) the two main bronchi of dogs were functionally separated.[1] In these experiments the left lung was used for production of atelectasis, either by aspiration of its gas or by blocking its cannula, while the right was used for ventilatory studies.

Ventilation of the right lung was measured by attaching the right bronchial cannula to a gas flow meter, which recorded the breathing frequency and minute volume every two minutes and from which the tidal volume could be calculated. During left lung atelectasis the total ventilation became equivalent to the right lung ventilation. Total ventilation prior to atelectasis was estimated by assuming that the right lung measurement was 60 per cent of the total ventilation when both lungs were open.

Right lung tidal volume changes occurring immediately upon blocking the left bronchus were measured from a spirometer record. A Drinker respirator was used to produce artificial respiration in certain animals paralyzed by succinylcholine or dead, in order to study right lung tidal volume changes when the left bronchus was blocked under those conditions.

Arterial blood *p*H measurements were recorded continuously from a Beckman *p*H meter blood glass electrode supplied by blood from a femoral arterial catheter and drained by a femoral vein catheter. This assembly rested in a 38°C. water bath.

DISCUSSION OF RESULTS

A series of 11 animals showed the following increases in right (open) lung ventilation when the left lung was rendered atelectatic. Measurements are the averages of at least 10 minute control and atelectasis periods.

The *vagal* effect in production of this hyperventilation was investigated by cold blocking (or cutting) both these nerves in the neck. Bilateral vagal block was obtained by circulating iced water through small copper coils

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enclosing the carefully dissected nerves in the neck. This block could be reversed by passing 40°C. water through the coils.

In five animals the vagus nerves were first blocked before producing left atelectasis. The right lung ventilatory changes were qualitatively similar to those occurring with atelectasis when the vagi were intact.
This indicated that the vagus nerves alone are not necessary for the production of hyperventilation by atelectasis.

A series of five animals was next tested by blocking the vagi *after* atelectasis had produced hyperventilation. The respiratory changes were similar

Table 1. Average Ventilatory Changes in 11 Dogs with Sudden Left Lung Atelectasis

	RATE/MIN	TIDAL, CC.	TOTAL VENT.,* CC.	pH
Control	31.7	72 8	3465*	7 37
Atelectasis	37 4	150 0	5180	7.35
% Increase	+18%	+105 8%	+49 4%	—.02

* Total ventilation during left atelectasis is equivalent to right lung ventilation. During control periods it is the ventilation of both lungs.

to those produced by vagal block when atelectasis was not present. Thus the rate decreased by 34 per cent (compared to 19 per cent when the left lung was open). Tidal increased 78.6 per cent (36.1 per cent when left lung was open) and total ventilation increased 15.3 per cent (7.1 per cent with left lung open) pH increased with vagal block under both conditions, +.041 with left lung collapsed and .033 with it open. These findings further minimized the role of vagal reflexes in the production of hyperventilation by atelectasis

The *blood chemical* effects associated with atelectasis were investigated

Table 2 Average Right Lung Ventilatory Changes Produced by Left Atelectasis When Vagi Are Intact and Blocked

VAGAL CONDITION	NO	RATE/MIN.	TIDAL, CC	TOTAL VENT, CC
Intact	11	+18 0%	+105 8%	+49 4%
Blocked	5	+17 9%	+ 92 7%	+85 4%

by occluding the blood flow through the atelectatic lung, thereby stopping the flow of blood through non-aerated lung Thus the arteriovenous shunting of non-ventilated blood should be stopped, arterial O^2 saturation improved, and arterial pCO_2 decreased, thereby eliminating part of the blood chemical stimulus to hyperventilation.

At the time of thoracotomy for placement of the endobronchial tube a series of heavy silk ties was looped about the left main pulmonary artery and led out anteriorly through the chest wall Two loops of silk were also passed about the artery on either side of these ties and secured to the posterior chest wall. Now when a loose tie was pulled up tightly through the chest wall it occluded the artery against the ties fixed posteriorly. The tie could be released by cutting and removal. The artery could thus be occluded several times in the same animal. Thirteen studies have been completed in four dogs. The averages are shown in Table 3

The records uniformly indicated that a ventilatory change occurred when the pulmonary artery was blocked. Table 3 indicates that this occurred by *a decrease in frequency of respiration* rather than by any real change in the tidal volume of the open lung.

The *"mechanical factor"* is the term applied to the forces which cause an immediate tidal volume increase in the open lung when the other lung is suddenly blocked off. Figure 1 shows the spirographic tracings of tidal

Table 3. Average Ventilatory Changes Produced in 13 Experiments on 4 Dogs by Pulmonary Arterial Occlusion in the Presence of Atelectasis

CONDITION	RATE/MIN.	TIDAL, CC.	TOTAL VENT., CC.
Artery open	38.4	148	5693
Artery blocked	34.9	145	5061
% Change	−9.1%	−2.2%	−9.7%

volumes reading right to left with inspiration upward. After the first three normal right lung tidal volumes appears an arrow which indicates sudden left bronchial occlusion during the succeeding two respirations. These two respirations show approximately a 30 per cent tidal volume increase. The *b* portion shows recurrence of this phenomenon when the left lung was blocked at the end of every other expiration. In the *c* and *d* portions are right lung tidal changes produced when the left lung was blocked at the end of inspiration.

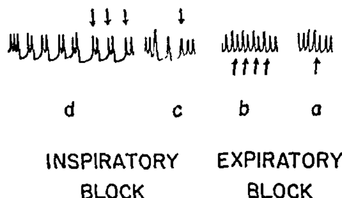


Fig 1. Spirometer record showing immediate increase in right lung tidal volume when left main bronchus is blocked. Inspiration is upward *a* shows result when left lung was blocked (arrow) at end of expiration, *b* when left lung was alternately blocked at expiration, and open, *c* when left was blocked at height of inspiration, and *d* left lung alternately blocked at inspiration and open.

Table 4 indicates the average changes in a series of 13 animals.

The phenomenon is always reproducible. It occurs despite vagal block or cutting and can be observed to occur in a dog paralyzed by succinylcholine or even one that is dead, if the respirations are artificially produced by a respirator

This effect of airway block can be seen in several reports of animal experiments centered on other aims,[3] and has been observed in humans during bronchospirometry.[2, 4] Its rapid and uniform occurrence makes blood chemical stimulation seem unlikely. Although vagal stimulation has been pre-

viously suggested,[4] our experiments with vagi blocked and in the dead animal rule against reflexes as the *sole* origin of the contralateral tidal increase.

We have, therefore, sought an explanation in a change of the elastic compliance of the thorax during left bronchial occlusion. Pressure-volume

Table 4. Average Tidal Volume Increase of Open Right Lung Immediately after Left Bronchial Block

LEFT BRONCHUS BLOCKED ON	NO	R L. TIDAL VOL BEFORE LL BLOCK	R L. TIDAL VOL AFTER LL BLOCK	% CHANGE
Expiration	13	101 5 cc.	133 7 cc	+30 6%
Inspiration	6	103 2	139.9	+35 5%
Expiration with vagi blocked	4	190 0	227.4	+19 7%
Expiration, dead dog	5	157 4	173 3	+10 6%
Inspiration, dead dog	4	137 5	154 0	+11.1%

curves for the individual lungs of dogs have been constructed when both lungs were open and alternately when the left was blocked and the right open

Figure 2 is the plot of the average right lung curves in 5 experiments

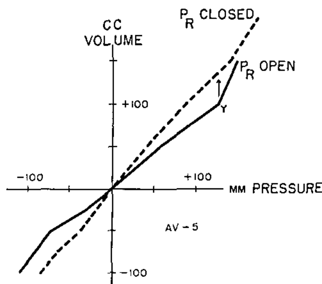


Fig 2 Average right lung relaxation pressure curves of 5 experiments. Solid line represents right lung P_R curve when left lung was open Broken curve represents right lung P_R curve when left main bronchus was blocked

The solid curve "P_R open" represents the relaxation pressure curve of the right thorax when the left lung was open. It is constructed by measuring the right lung volume change (V axis) occurring at a given constant pressure differential (P axis) produced by a Drinker respirator (so that no muscle action occurs). For example, on the P_R open curve the right lung

increased 81 cc. in volume when the Drinker respirator exerted a constant −100 mm. H_2O pressure (the minus Drinker pressure being equivalent to positive pulmonary pressure of 100 mm.).

The broken curve "P_R blocked" was constructed from alternate right lung measurements made with the left bronchus blocked at the resting phase after expiration. This new curve is shifted upwards and indicates a greater compliance, i.e., a larger volume change for the same pressure differential. The shift of the curve is actually a change of the compliance of the chest wall, since it can be shown that the lung compliance remains unaltered.

Figure 2 indicates that the 2 compliance curves are essentially linear and that the compliance with the blocked lung is about 35 per cent greater over this range. Thus one would predict that for the same force applied a 35 per cent increase in tidal volume is to be expected by blocking the left lung on expiration. The tidal volume on the P_R open curve shown at Y in Figure 2 should shift (arrow) to the increased tidal volume on the P_R closed curve. That this is nearly the value obtained during spontaneous breathing may be seen in Table 4 (30.6 per cent increase with expiratory block). The spontaneous changes which occur after block at inspiration can be explained on a similar basis.

The increased compliance which follows upon occlusion of the contralateral side can be explained on the basis of the mediastinal shift which occurs upon block. When both lungs are open the mediastinum remains essentially fixed and therefore does not contribute to the elastic component of the chest and diaphragm. However, as soon as the left lung is blocked on expiration it will move toward the left side during the ensuing inspiration and absorb an additional volume of air (approximately 35 per cent of the original right tidal volume) when the right lung expands to the same force as applied before block.

The changes in tidal volume after lung blocking are further modified by reflexes mediated over the vagus nerves and blood chemical changes as discussed above. However, the altered mechanical properties remain as one of the essential factors in explaining the increase in tidal volume.

SUMMARY

Unilateral pulmonary atelectasis uniformly produces hyperventilation of the open lung. Three factors may influence this response. The role of the vagi is minimized, since blocking them does not abolish the open lung response to atelectasis and since vagal block produces a similar pattern whether or not atelectasis has occurred.

Blood chemical factors acting on the central respiratory center probably contribute the main stimulus to hyperventilation during atelectasis. Some decrease in ventilation could be produced by blocking the shunt of blood through non-aerated lung.

A mechanical shift of the elastic forces of the chest occurs immediately upon blocking one lung and explains an immediate increase in tidal air volume of the open lung. This seems to be due to a change in chest wall compliance which becomes altered by the shift of the mediastinum. This factor is probably a function of the amount of blocked pulmonary space. It invariably and immediately increases the tidal exchange of the lung remaining open.

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BRONCHOSPASM DUE TO INCREASED CARBON DIOXIDE IN INSPIRED AIR; A VAGAL REFLEX*

RICHARD M. PETERS

During an investigation of pulmonary vascular resistance, it was noted that when ventilation with gas mixtures with increased concentration of carbon dioxide was carried out, dogs' lungs became overdistended. Starling pumps, which have a positive inspiratory and passive expiratory phase, were being used for artificial ventilation. The dog was therefore forced to accept an inspiratory volume which he could not exhale. The assumption was made that this troublesome overdistention was due to bronchospasm. The studies to be described in this paper were undertaken to elucidate this problem.

METHOD

Mongrel dogs were anesthetized with chloralose (100 to 125 mg per kilogram) after having received morphine (1 mg. per kilogram) one-half hour previously. The chest was widely opened through a left thoracotomy, and in most dogs both phrenic nerves were crushed In some dogs a simple tracheal cannula was used; in others, a double lumen cannula was tied into the trachea and left main bronchus In the latter group, care was taken not to obstruct any of the secondary bronchi and to place the tie above the level of the vagal branches to the left lung The lungs were ventilated by Starling pumps. In those dogs with double lumen cannulae in place, the lungs were ventilated with separate Starling pumps so that gas mixtures of different concentration could be offered to each side.

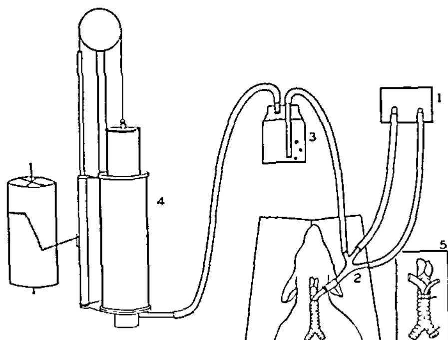
Two methods were used to determine the presence of bronchoconstriction In the first, the pressure swings associated with the respiratory cycle were recorded by aneroid or Sanborn electromanometers through a 14 gauge needle inserted into the tubing connecting the pumps to the dogs The rate and volume of the pumps were adjusted to give maximum positive pressures of about 15 mm. Hg while the dog was ventilated with air. Any rise in pressure represented a change in bronchial resistance.

The second method was a modification of the Konsett[1] method (Fig 1). A side arm was added to the Y tube connecting the tracheal cannula to the

* From the Department of Surgery, University of North Carolina School of Medicine, Chapel Hill The study was supported by a United States Public Health Service Grant-in-Aid Grant No H-1257C.

pumps. A tube was led from the side arm to a water trap and the escape side of the water trap was connected to a spirometer. The stroke volume of the pumps was set to make the maximum pressure just equivalent to the

latter method is more sensitive than the simple measurement of changes in pressure. In those animals in which the double lumen cannula was used, only the right lung was recorded by the second method. The gas mixtures employed contained 19 to 21 per cent oxygen and 10, 20 or 30 per cent carbon dioxide. The balance was nitrogen. In two dogs, mixtures of 5, 10



choconstriction by modification Konsett ...m, 3, water trap, 4, recording spirome- ... For description, see text.

or 15 per cent carbon dioxide in oxygen were used to determine the lower limit at which bronchoconstriction would occur.

Arterial blood was drawn (1) when equilibrium between respiratory gases and blood gases had been achieved, (2) at the onset of bronchoconstriction, (3) at the end of bronchoconstriction. With these samples, the following determinations were made. Whole blood oxygen content and saturation was determined by the spectrophotometric method of Hickam,[2] pH with the Cambridge glass-electrode pH meter at room temperature and corrected to body temperature by subtracting 0.014 pH units per degree difference in temperature, and the plasma carbon dioxide content in the Van Slyke manometric apparatus. The plasma pCO_2 was calculated from the latter two determinations by the Henderson-Hasselbalch equation.

When the above studies were completed, bronchoconstriction was again induced. Atropine in doses of 0.02 to 0 029 mg. per kilogram was given to some animals, and cervical vagotomy was performed on others.

RESULTS

When both lungs were ventilated with gas mixtures containing 10 per cent carbon dioxide, bronchoconstriction was consistently induced as measured by the Konsett method, and in most instances by the pressure method. When only the left lung was ventilated with 10 per cent carbon dioxide, no bronchoconstriction ensued except in dogs 26 and 30, but it consistently occurred when 20 per cent carbon dioxide was substituted. In order to differentiate the effect of elevated arterial pCO_2 level from that due to

Table 1. The Arterial pCO_2 Levels at the Start, End and during the Course of Bronchospasm Due to Increased Concentrations of Carbon Dioxide in the Inspired Gas Mixtures

DOG NO.	PERCENTAGE CONC. CO_2 IN INSPIRED GAS	LUNG RESPIRED WITH CO_2	BRONCHOSPASM	START OF BRONCHO-CONST.	END OF BRONCHO-CONST.	EQUILIBRIUM WITH RESPIRED GASES
23	20	Right	Present			98
	30	Right	Present			124
24	20	Left	Present			113
25	30	Right	Present			129
26	10	Left	Present			45
28	20	Left	Present			81
30	10	Right	Present			47
	10	Right	Present			60
40	30	Left	Present			71
41	10	Both	Present	59 & 66		79
	20	Left	Present			65
	20	Both	Present			146
42	10	Both	Present	68		71
	20	Left	Present		34	76
43	20	Left	Present	84		
44	5	Both	Absent			33
	10	Both	Present	59	32	
	5	Both	Absent			40
	15	Both	Present	68	38	93
45	5	Both	Absent			44
	10	Both	Present	56		
	10	Both	Present	55	33	
	5	Both	Absent			43
	15	Both	Present	74	36	

elevated alveolar pCO_2, both lungs were ventilated with 10 per cent CO_2 and later only the left lung with 20 per cent CO_2 The arterial pCO_2 was found to be essentially the same under these two circumstances. Recordings made of the response of the right lung show no difference in the two circumstances. Ventilation with 5 per cent CO_2 in both lungs caused no bronchoconstriction At the moment of onset of bronchoconstriction, the pCO_2 levels varied from 56 to 84 mm. Hg (Table 1). In dogs 26 and 30, bronchoconstric-

effect lasted for more than one hour. Vagotomy likewise promptly relaxed the bronchoconstriction.

The level of arterial pCO_2 appeared to affect the degree of bronchoconstriction. By the less sensitive pressure method in some animals, a significant response did not occur on 10 per cent CO_2 in the inspired gas but did with 20 per cent or 30 per cent CO_2. Representative curves are shown in Figure 2.

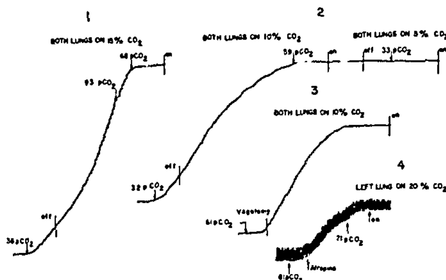


FIG. 2. These are representative curves from spirometer recordings, reading from right to left. The start of bronchospasm is represented when the curve descends, showing an ... howing no ... levels and ... broncho- ... atropine

DISCUSSION

Roy and Brown[3] in 1885 demonstrated that asphyxia caused bronchoconstriction. Einthoven,[4] also in 1885, reported that inhalation of gas containing high concentrations of carbon dioxide caused bronchoconstriction which was abolished by section of the vagus nerves. In 1903, Dixon and Brodie[5] confirmed this finding in cats. Daly et al.[6] also found that perfusion of the brain with blood high in carbon dioxide or low in oxygen caused bronchoconstriction which was reversed by section of the vagus nerves or injection of atropine. In isolated perfused lung experiments, Nisell[7] found that carbon dioxide excess or oxygen lack caused bronchodilation. If Nisell's observations in this artificial preparation are applicable to the intact animal, the local effects of increased concentrations of carbon dioxide in the lungs must be opposite to the central effect.

Daly questioned the importance of the central reflex, since he considered the carbon dioxide levels he used to be above physiologic ranges. The highest concentration of carbon dioxide used was 10 per cent, which would give an arterial pCO_2 of approximately 76 mm. Hg. It has been demonstrated, however, that levels as high as or higher than this are frequently encountered during thoracotomies (Beecher,[8] Taylor,[9] Roos,[10] and Stead[11]), and in

patients with pulmonary insufficiency. If during the course of an operative procedure the pCO_2 is allowed to reach levels which might cause bronchoconstriction, ventilation would be made more difficult. This is clinically manifest to most anesthetists and thoracic surgeons in the problems encountered in respiring patients adequately subsequent to prolonged apnea during induction of anesthesia, prolonged endotracheal suction, or inadequate ventilation for some other reason. A demonstration of whether heavy atropinization in the extreme cases might be in order will have to await a suitable clinical situation.

If this reflex is still active in patients with emphysema and chronically elevated arterial pCO_2 levels, the prolonged improvement achieved by a period of artificial ventilation might be explained, in part, by the decrease in the degree of bronchoconstriction associated with the lowering of the arterial pCO_2.[11]

It is also interesting to speculate about the role of this reflex in paroxysmal nocturnal dyspnea. It is possible that an exaggerated nocturnal rise in arterial pCO_2 level might serve to trigger the chain of events. This is under investigation by a study of the circulatory as well as bronchomotor effects of changes in systemic blood gases and respiratory gases.

In the intact animal, it is hard to change the respiratory gases without significantly altering the systemic arterial gases, and vice versa. Nisell's and Daly's contrasting results have not been clarified by this investigation. If Nisell's observation is valid, the central reflex is much stronger than the opposite local one. If the bronchoconstriction response of the right lung following the ventilation with 20 per cent CO_2 in only the left lung had been greater than with 10 per cent CO_2 in both lungs, this would be indirect confirmatory evidence of Nisell's experiment. The failure to show such a difference may be due to lack of sensitivity of this method. At present, studies are under way in which the blood from the ascending aorta is passed through a donor set of lungs and then back to the systemic circulation by means of a perfusion pump This technique will permit independent variation of the systemic blood gases and respiratory gases, and perhaps these two effects may be differentiated.

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AN EVALUATION OF ANTIEMETIC DRUGS IN THE CONTROL OF POSTOPERATIVE NAUSEA AND VOMITING*

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Patients from several surgical services were designated at random when ether was to be used as the anesthetic and arbitrarily assigned to the control group or one of the treated groups. An antiemetic work sheet was appended to the chart and remained with the patient throughout his stay on the recovery ward. The details of the anesthesia were recorded by the anesthetist and the postoperative events were noted by the recovery ward personnel. In the treated groups, the drug, dose, time, and route of administration were recorded and side effects were listed. All drugs were given intravenously by adding them to the drip chamber of the infusion set in the proper dosage to insure a uniformly slow administration. At a rate of 60 drops a minute, this provided for administration of the drug in gradually decreasing concentration over about a 20 minute period. The treatment was started about 20 minutes before the contemplated completion of surgery. Recovery ward attendants listed the vomiting episodes individually and included the time of onset and duration. Incomplete and incorrect work sheets were eliminated. Obviously, this system of observation included a large number of observers and accounted for some variation in interpretation and completeness of the records.

Operations peculiar to combat casualties were relatively infrequent. The majority of the procedures were those seen in any large civilian hospital. To minimize possible complications from antiemetic therapy, patients over 60 years of age, children under 100 pounds, poor risk patients, and intracranial cases were excluded from the study. Examination of age of the patient and duration of operation revealed a homogeneous population for the control and treated series (Table 1).

The antiemetic drugs used in this study included diphenhydramine hydrochloride, cyclizine lactate, promethazine hydrochloride, and chlorpromazine hydrochloride.

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Both diphenhydramine and cyclizine contain the benzhydryl rings. Chlorpromazine is the chlorinated isomer of promethazine. β-dimethyl amino ethyl benzohydryl ether -8-chlorotheophylinate is the 8 chlortheophylline salt of diphenhydramine. Chinn[1] has shown that the 8 chlortheophylline portion of β-dimethyl amino ethyl benzohydryl ether -8-chlorotheophylinate is ineffective in preventing motion sickness. Therefore, diphenhydramine alone was used because of its greater solubility.*

Table 1. Characteristics of Control and Experimental Populations

	CONTROL NO.	DIPHENHY-DRAMINE	CYCLIZINE	PROME-THAZINE	CHLORPRO-MAZINE
Number	353	133	167	39	54
Mean age	36 6	35 9	34.8	34.7	32 48
Standard deviation	15 35	13 0	12 5		10.75
Mean duration (min.)	179 44	183 8	185 6	190.7	171 54
Standard deviation	79 6	78 1	79 8	76.8	67.11

These drugs are related to the antihistamines, but only diphenhydramine has any appreciable effect in this respect. The effectiveness of diphenhydramine in 100 mg. intravenous doses in preventing postoperative vomiting has been established[2] The dosages used were arbitrary and may not represent the optimal amounts since they were selected primarily on an estimated freedom from side effects. The doses were:

diphenhydramine	100 mg
cyclizine	50 mg.
promethazine	50 mg.
chlorpromazine	25 mg.

Table 2 Percentage of Patients Vomiting or Reporting Nausea

	CONTROL	DIPHENHY-DRAMINE	CYCLIZINE	PROME-THAZINE	CHLOR-PROMAZINE
Patients vomiting one or more times, %	61 8	42 9	41 0	41 0	50
Patients reporting nausea, %	59 5	48 7	50	62 85	46 9
Mean number of vomiting episodes per patient	3 60	2 44	2 49	2 0	3 25
Patients vomiting 5 or more times (of those who vomited at all), %	23.7	7 1	9 9	0	22 2

The period of observation for most patients included the first 12 hours after operation and in many instances covered a 24 hour period. Immediate postoperative patients are poor candidates for subjective analysis, and often it appeared that suggestion by questioning was enough to elicit nausea in an otherwise uncomplaining patient. Less reliability, therefore, was attached to reported presence or absence of nausea. The work sheets also included information as to the type and duration of the anesthesia and the

* The dru[illegible] ienhydramine—Parke Davis & Co, [illegible] Co, Tuckahoe, New York; prome[illegible] lorpromazine—Smith, Kline & French Lab, Philadelphia, Pennsylvania

various adjuvant drugs used in the correlation of anesthetic factors causing vomiting. Information as to the extent and nature of the side effects of the antiemetic drugs was also sought.

RESULTS

There were 353 patients in the control group, 133 in the diphenhydramine group, 167 in the cyclizine group, 39 given promethazine and 54 in the chlorpromazine series. The over-all suppression of vomiting by the antiemetic drugs was incomplete but highly significant.

A plot of time of occurrence of vomiting showed increasing frequency of emesis during the first 120 minutes after operation. In the control series there were 85 vomiting episodes per 100 patients during the first 120 minutes of the postoperative period. This was decreased by the antiemetic drugs as follows: diphenhydramine, 33 per 100, cyclizine, 31 per 100; promethazine, 21 per 100; and chlorpromazine, 21 per 100. Thus, from one-half to

Table 3. Percentage of Patients per Number of Vomiting Episodes

NO. OF VOMITING EPISODES/PATIENT	CONTROL	DIPHENHY-DRAMINE	CYCLIZINE	PROMETHA-ZINE	CHLORPRO-MAZINE
	%	%	%	%	%
0	38 2	57 1	58 0	59.0	50.0
1	11 0	18.0	14.4	20 5	7.41
2	15 9	7 5	10 2	10.2	14.8
3	9.9	7 5	10.2	0	9.26
4	9 35	4 5	3 0	10.2	7.4
5	5 4	2 3	1.8	0	3.7
6	3.4	2 2	0.6	0	5 5
7 or more	6 8	0 7	1 8	0	1.8

three-quarters of the patients who would have vomited did not do so in the treated series. In the control group, 48 per cent of the vomiting occurred during the first 120 minutes after surgery and 71 per cent in the first 180 minutes postoperatively.

Obviously, these antiemetic drugs have a limited duration of effect* and a study is now under way on the effectiveness of interval intravenous and intramuscular dosage during the first 12 postoperative hours.

Various factors associated with the anesthesia and surgical procedures may be related to the severity of vomiting. The combination of these factors may, in the future, be correlated with the indication of need and/or intensity of antiemetic therapy. Table 4 lists several technical factors concerning the anesthesia in the control series and indicates no significant variation in emetic episodes for those factors listed.

The incidence of vomiting increased directly with the duration of anes-

* he control series. ten not indicated 20 minutes was the first critical there were 13 2 30 + 20) post- ., 7 per 100 for promethazine, 6 per 100 for chlorpromazine.

thesia and operation (Table 5). Smith[3] reports an increase from 20 per cent to 47 per cent vomiting when the operation time was increased from 30 to 120 minutes. He also recorded a higher incidence in female patients.

The narcotic used as preanesthetic medication has also been implicated as a factor causing nausea and vomiting.[4] In our series, morphine and

Table 4. Vomiting as Influenced by Technical Factors

	NO PTS	INCIDENCE OF VOMITING %	AVERAGE NO. VOMITING EPISODES/ PT. WHO VOMITED	PATIENTS VOMITING 5 OR MORE TIMES OF THOSE WHO VOMITED %
Over-all	353	61 8	3 60	25.2
Pentothal used for induction	225	64 0	2 14	14.2
Nitrous oxide used for induction	55	63 6	2 61	20 0
Nitrous oxide and Pentothal induction	64	51 6	1 70	14 5
Muscle relaxant adjuvant drugs	111	62 1	1 93	10 0
Endotracheal tube used	235	61 7	2 09	13 8
No endotracheal tube used	118	60 1	2 37	32 4
Females	157	77 7	3 50	32 0
Males	196	49 4	3 06	17 5

meperidine with scopolamine and atropine respectively are compared in the control group.

It is obvious that the dose response relationship to emetic episodes for morphine is not significant, while that for meperidine is.

Although the numbers of patients for each type of operation was not large

Table 5. Relation of Duration of Operation and Vomiting Incidence in the Controls

DURATION	NO PTS	INCIDENCE OF VOMITING %	AV NO VOMITING EPISODES	VOMIT 5 OR MORE TIMES %
Less than 90 min	34	26.7	2 5	0
Less than 180 min	185	54 0	3 12	22 1
180 min or more	134	69 1	4 04	28 2

enough for precise analysis (Table 7), certain trends may be noted (Table 8).

Clinical evaluation on an individual basis indicated a higher incidence of vomiting with deeper anesthesia, hypoxic episodes, and fluctuating planes of anesthesia, but these factors were often not completely recorded on the

work sheets and no attempt has been made to correlate them statistically.

Side Effects. It is unfortunate, in respect to studying their side effects, that the antiemetic drugs were administered shortly before the completion of surgery. At this time the anesthetist is occupied with the care of the patient and in many instances the record of the observations is minimal.

Table 6. Relation of Preanesthetic Analgesics to Vomiting

	NO. PTS.	INCIDENCE OF VOMITING %	AVERAGE NO. VOMIT PER PT. WHO VOMITED	PTS. VOMIT 5 OR MORE TIMES (OF THOSE WHO VOMITED) %
Morphine Sulfate:				
M.S. 4 mg.	2	50	2 0	0
M S. 6	20	55	1 6	45
M.S. 8	128	65 6	2 63	34 3
M S. 10	152	58 5	1 96	11 4
M.S 12	2	50	2 0	0
Total patients given M.S.:	305	61 3	2 21	16 1
Meperidene HCl:				
Mep. 35 mg.	2	50	3 0	50
Mep. 50	12	50	1 91	16 6
Mep. 75	19	73 7	1 78	26 3
Mep. 100	10	90 0	3 5	30
Total patients given Mep.:	43	72 1	2 28	16.3

Table 7. Number of Patients Having Each Type of Operation

OPERATION	NO.	OPERATION	NO.
Genitourinary	31	Abdominal and general	84
Gynecologic	49	Thoracic	46
Orthopedic	42	Plastic	33
Neurosurgical*	32	Miscellaneous	14
Eye, ear, nose and throat	22	Total	353

* Neurosurgical includes sympathectomies, rhizotomies, laminectomies (not specified) and discs, but craniotomies have been deleted

Table 8. Operations Grouped by Incidence of Emesis

HIGH INCIDENCE OF EMESIS		LOW INCIDENCE OF EMESIS	
Hysterectomy	18 per 20	Gastrectomy	1 per 6
Laminectomy	21 per 30	Intestinal resection	4 per 10
Fenestration	3 per 3	Cardiac operations	6 per 15
Appendectomy	5 per 5	Other Thoracotomy	11 per 25

The method provided for a slow intravenous administration, and in many instances the cardiovascular effects took place between the operating room and the recovery ward and therefore were not recorded. Precise analysis of these reactions is therefore unreliable By and large, there were no unusual or undesirable side effects. In about one-quarter of the cases there were effects following the administration of the various drugs.

Shock is included in Table 9, not because it is thought that any of these drugs are the cause, but because in several instances of postoperative shock the effects were apparently intensified by the drugs. Patients who had inadequate volume replacement during surgery and were in borderline or "compensated" shock occasionally had the clinical picture of shock more acutely focused by the administration of the drugs mentioned. Their response to proper treatment was satisfactory.

Table 9. Side Effects of Antiemetic Drugs

	CYCLIZINE	DIPHENHYDRAMINE	PROMETHAZINE	CHLORPROMAZINE
Prolonged drowsiness	Rare	Occasional	Frequent	Frequent
Analgesia (potentiation of narcotics?)	Slight	Slight	Slight	Greater
Hypertension >150/90	Rare	Rare	Occasional	Rare
Hypotension <80/60	Rare	Rare	Rare	Occasional
Tachycardia >120	Occasional	Occasional	Frequent	Frequent
Bradycardia <60	Rare	Rare	Rare	Rare
Shock	0	0	Rare	Occasional
Muscle fibrillation (or jactitations) immediately after injection	0	0	Occasional	Rare
Skin rash	Rare	0	Rare	0
Phlebitis at injection site	0	0	Rare	0

Promethazine given at 100 to 150 mg. dosage intravenously is reported to be followed by a phase of agitation similar to that of stage II nitrous oxide anesthesia.[5] With the dosage we used there was rarely any indication of central nervous system stimulation.

SUMMARY

1. The incidence and severity of vomiting after ether anesthesia and operation was studied in a group of 353 patients and found to occur in 61.8 per cent of the patients with a mean of 3.6 episodes per patient. Various technical factors of anesthesia were investigated as to their influence on this incidence

2 Four antiemetic drugs given in a single dose intravenously prior to the completion of the operation were found to decrease this incidence.

3. The side effects of these drugs were recorded

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USE OF THE RECORDING OXIMETER AS A MEANS OF EVALUATION OF PULMONARY RESERVE*

W. E. ADAMS, J. F. PERKINS, JR., MIGUEL CASTELLANOS, ADOLFO FLORES, AND RALPH F. CARLSON**

In the management of pulmonary disease the clinical importance of the status of pulmonary reserve has long been appreciated. An accurate assessment of pulmonary function permits planning for safer management, and the making of a more accurate diagnosis and prognosis. Thus, in pulmonary surgery where a choice is permissible, the amount of lung tissue to be safely resected may be more accurately determined.

In recent years much has been accomplished toward a solution of this problem by a large group of workers. Since pulmonary function depends upon the summation of several parts, i.e., ventilation, mixing, diffusion and circulation, tests devised for its assessment have of necessity been many and varied. In the past these tests have concerned themselves chiefly with the transportation, mixing and diffusion of gases within the lungs. The factor of circulation, and especially the influence on the heart of deficiencies in pulmonary function, have received less attention. Cor pulmonale as a complication of certain types of diseases of the lungs has long been recognized. However, a study of the factors involved in its development as well as its production in experimental animals has been given little consideration.

With the rapid development of pulmonary surgery during the past two and one-half decades, many complications contributing to the mortality have been studied and largely eliminated. The condition termed "lowered pulmonary reserve" still remains as a serious obstacle in pulmonary surgery for the aged, or for those patients with chronic bilateral involvement.

In 1950 studies dealing with the dynamics of the lesser circulation both in normal dogs and in dogs with reduced pulmonary capacity were carried out in our laboratory.[1,2] The right ventricular pressure was found to be elevated, somewhat in proportion to the amount of lung tissue collapsed or resected. Many animals tolerated a reduction in lung capacity to 25 per cent of normal, but *only one-fourth of the dogs survived reduction to as little as* 15 per cent of normal. Right heart pressures were elevated as much as 100 per cent in the former group, and even more in dogs whose lung tissue was reduced to only 15 per cent of normal. Death of these animals usually occurred within five days, and the autopsy findings corresponded with those seen in cardiac failure.

In a subsequent study,[3] alterations in right heart pressure were correlated with arterial blood oxygen saturation in dogs having reduction in lung capacity. Oxygen saturation studies were made using the Wood earpiece and a continuously recording oximeter, which was devised and built in these laboratories (by J.F.P.)[4] and checked by Van Slyke determinations. These

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studies were made after the extirpation of one lung in dogs in which occlusion units for sudden further reduction of lung function to 15 per cent had been placed. These studies revealed that although pulmonary hypertension of serious import (100 to 150 per cent increase) developed when lung function was reduced to 15 per cent of normal, the accompanying fall in arterial blood oxygen saturation was small (average 5.0 per cent). It was therefore concluded that pulmonary hypertension was a significant factor as a cause of death in the presence of reduced pulmonary reserve.

In view of the above findings, studies were begun in humans making use of the recording oximeter to determine the status of pulmonary function following major thoracic operations.

The present-day practice of administering high levels of oxygen flow per minute through a nasopharyngeal catheter to all patients after surgery on the thorax, although increasing the oxygen content of the blood, is not exempt from causing ill effects to the patient. Drying of mucus membranes, with the production of tenacious secretions in the upper respiratory tract and the mechanical effects on the mucosa, deters proper elimination of these secretions and predisposes to atelectasis and pneumonia. This in turn may be accompanied by a further reduction in pulmonary capacity.

In the past, changes in pulse, respiratory rate and the general clinical condition of the patient were the criteria employed to determine the need for the induction, maintenance and discontinuation of oxygen therapy. It was considered advisable to administer postoperatively between 5 and 10 liters of oxygen per minute through a nasopharyngeal catheter, and any flow under 5 liters per minute was thought to be of little value. Although pulse rate and respiratory activity are signs of considerable value in de- they are not as accurate and reveal- levels of arterial oxygen saturation. rding oximeter in the study of clinical problems in this institution provided an accurate method for the determination of the indications for, and the effectiveness of, various techniques of oxygen therapy after thoracic surgery.

METHODS

Fifty patients on whom different thoracic surgical procedures were performed were evaluated in this study. Among the surgical procedures were pneumonectomy, lobectomy, segmental resection, thoracoplasty, aortic resection, mitral valvulotomy and esophageal operations. All of these patients had reduced efficiency in pulmonary ventilation, due in part to an operation on the chest wall Those patients who underwent pulmonary resection had the additional factor of reduction in lung capacity. The speed of the recording paper was set at 1 cm. per 2.5 minutes in order to obtain several hours of continuous recording.

Immediately postoperatively the patients were transferred to the recovery room, where observations on the pulse rate, respiratory rate, arterial oxygen saturation and the general clinical condition were carefully recorded on the oximeter record.

A base line for arterial oxygen saturation, pulse rate and respiratory rate was then established by allowing the patient to breathe room air long enough to get a plateau for at least five minutes.

One hundred per cent oxygen was then administered by means of (1) a face mask connected to a McKesson anesthesia machine and inhalation positive pressures of 10 mm. Hg applied, and (2) a B.L.B. face mask with an oxygen flow of 10 to 12 liters per minute.

As soon as the patient regained consciousness, oxygen was administered through a nasopharyngeal catheter. Previous to this time the patient's tidal volume was insufficient to allow the use of such a catheter. Flows of 1.0, 2.5, 5.0, 7.5 and 10.0 liters per minute of oxygen were then established, sufficient time being allowed between changes of rate of flow to permit securing of sustained levels of saturation. The efficiency of the oxygen therapy was judged mainly on the basis of arterial oxygen saturation values, and the patient was left on the lowest oxygen flow per minute required to maintain adequate arterial oxygen saturation.

The patient was followed thereafter in his room and rechecked as often as necessary in order to determine his oxygen requirements. Determinations of arterial oxygen saturation were made at least once every 24 hours using the same method as described above.

RESULTS

In this series, 90 per cent of the 50 patients showed a postoperative decrease [illegible] ing from three hours to several weeks [illegible] ice of cardiorespiratory complications. [illegible] procedure the average saturation for all patients was 88 per cent, whereas normal figures range between 96 to 98 per cent.

This postoperative hypoxia responded quickly to oxygen therapy regardless of the method of its administration. When given 100 per cent oxygen with a McKesson inhalation positive pressure machine, above normal values of arterial oxygen saturation were obtained within 1 or 2 minutes. In 20 patients the average increase of arterial oxygen saturation was 10 per cent, and a plateau was maintained at 98 to 99 per cent levels. Comparable results were obtained when using B.L.B. masks with oxygen flows of 10 to 12 liters per minute.

The results in an average case where oxygen was administered through a nasopharyngeal catheter are shown in Figure 1. It will be noted that a flow of 2.5 liters of oxygen per minute proved effective in raising the saturation to normal or close to normal levels in all patients without cardiorespiratory complications, whereas without this continuous determination of arterial oxygen saturation, a flow of 5 to 10 liters of oxygen per minute would have been considered necessary. The average increase in saturation obtained with a flow of 2.5 liters per minute in all 50 patients 24 hours following operation was 5 per cent, i.e., from 88 to 93 per cent. A flow of five liters of oxygen per minute resulted in a 7 per cent rise in saturation (from 88 to 95), or only 2 per cent higher than that reached using 2.5 liters per minute. Increasing the oxygen flow to 7.5 or 10 liters per minute did not appreciably increase the saturation level above that obtained in most patients given 5 liters of oxygen per minute.

A decrease in pulse and respiratory rates occurred following the return of arterial oxygen saturation levels to normal. In 50 determinations made 24 hours after surgery, the average decrease in pulse rate was found to be 10

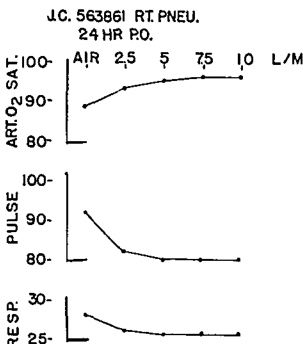


Fig 1. Response to oxygen administered through a nasopharyngeal catheter to a 62 year old patient twenty-four hours after a right pneumonectomy. Near to maximum rise in A O S. was obtained with an oxygen flow of only 2.5 liters per minute. Notice the changes in pulse and respiratory rate closely associated with the changes in arterial oxygen saturation.

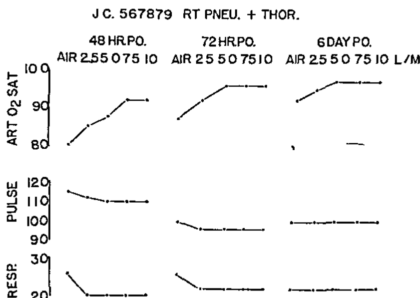


Fig. 2 Response to oxygen therapy in a 62 year old patient 48 hours, 72 hours and 6 days after a right pneumonectomy and a tailoring thoracoplasty Improved oxygen saturation corresponded with clinical improvement of the patient Notice correlation of pulse and respiration with arterial oxygen saturation figures.

per minute when the arterial oxygen saturation was raised to a normal level. Changes in respiratory rate, although not as clear cut as alterations in pulse rate, showed a decrease in most patients as oxygen saturation was increased.

In a small group of patients, particularly after pneumonectomy, in the presence of cardiorespiratory complications such as retained bronchial secretions, atelectasis and pulmonary edema, or in patients with marked emphysema, the use of inhalation positive pressure oxygen, B.L.B. masks or a high rate of oxygen flow (7.5 to 10 liters per minute through nasopharyngeal catheters) all proved equally effective in maintaining adequate arterial

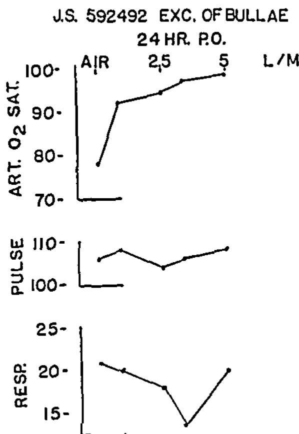


Fig. 3. Response to oxygen therapy in a 59 year old patient 24 hours after resection of the left lower lung lobe for emphysematous bullae. The saturation was raised 14 per cent on an oxygen flow of 1 liter per minute through a nasopharyngeal catheter. Notice lack of correlation in pulse and respiration with saturation values

oxygen saturation until these transitory situations were corrected with proper treatment (Fig. 2).

In some patients the use of only 1 liter per minute oxygen flow through a nasopharyngeal catheter raised the arterial oxygen saturation 10 to 15 per cent, thus demonstrating the importance of even small additions of oxygen. This finding was observed only in patients with marked decrease in pulmonary reserve. The same observation was made preoperatively in patients with advanced pulmonary emphysema. It was also seen following pneumonectomy in patients with severe complications such as atelectasis and

bronchopleural fistula. Patients with marked pulmonary hypertension during the postoperative period also showed a rapid response to small flows of oxygen (Fig. 3). The significance of this latter observation is under investigation at the present time.

SUMMARY AND CONCLUSIONS

The use of oxygen therapy is indicated as a routine procedure after all major thoracic operations. In young patients without cardiorespiratory complications, these studies show that there is likely to be no need for oxygen therapy after the day of operation. In older patients and in the presence of cardiorespiratory complications, oxygen therapy may be a very valuable part of postoperative therapy for a variable period of time.

but may be hazardous to the extent that it may lead to atelectasis with further reduction in pulmonary reserve.

The use of inhalation positive pressure oxygen and of a B L B. mask is indicated only until the patient regains consciousness or in the presence of complications. The amount of discomfort to the patient and the increase in both nursing care and expense support this view.

Without an oximeter, one must rely on the color of the patient and the pulse and the respiratory rate in the regulation of oxygen flow. According to this study, a good rule to follow is to reduce the flow of oxygen from 10 liters per minute to the point at which there is an increase in pulse rate and respiratory rate. This level is likely to be no more than 5 liters per minute.

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THE EFFECT ON THE CENTRAL NERVOUS SYSTEM OF INTERRUPTED CIRCULATION DURING REFRIGERATION ANESTHESIA*

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Adequate direct vision in a bloodless field has always been the desire of every surgeon. Until recent years, this wish could not be fulfilled in cardiac surgery. Even though it had been shown that the heart could withstand surgery almost as well as any organ, the ability to have direct vision and a bloodless field seemed to be impossible. The development of a by-pass procedure, i.e., the heart-lung pump, seemed to make this possible.[1-4] Arrest of circulation during refrigeration anesthesia made the possibility even more promising.[5-8]

Intracardiac operations with direct visualization now may be performed with either a heart-lung pump, or limited arrest of circulation while the organism is protected with refrigeration. The heart-lung pump is expensive, complicated, not readily available, and not yet entirely satisfactory. The interruption of the circulation during refrigeration is simple, and good clinical results with up to eight and one-half minutes of interruption have been reported.[9-11]

However, it is important to establish that a reasonable period of interruption of the circulation with refrigeration causes *no* central nervous system damage, if the interruption method is to be chosen instead of a heart-lung pump.

PROCEDURE

Twenty-eight unselected mongrel dogs, of varying ages, were used in the experiment (Table 1). Of these, one was used as a control for histologic study. Twenty-seven dogs had refrigeration anesthesia. Two dogs were sacrificed after only refrigeration, as controls. A total of twenty-four animals had arrest of circulation under refrigeration anesthesia. There were six deaths directly due to the operative procedure, an operative mortality of 25 per cent.

The operative procedure was carried out under intravenous human sodium pentobarbital. Human preparation was used because of the interference of the preservative in the veterinary preparation on the electroencephalogram. An endotracheal tube was inserted, and positive pressure oxygen used. After a preoperative EEG, the animal was placed in ice, and removed when the rectal temperature reached 83°F. After removal, the temperature fell up to 7° prior to circulatory arrest.

The operation *consisted of opening the right chest,* ligating the azygos system, and passing ligatures around the superior and inferior venae cavae and the aorta. The pericardium was opened widely. While an EEG was being taken, the ligatures were tightened on command. The aorta was

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occluded in the first four experiments. The pulse rate and appearance of the heart were closely observed.

At the end of the prescribed time, the superior vena cava and the aorta were gradually opened. Two minutes later, the inferior vena cava was opened After observation of the heart for fifteen minutes the pericardium was repaired, and the chest closed. A chest tube, attached to a water seal, was used.

The animal was then placed in a water bath at 45°C. and kept there until the rectal temperature was 99°F. The dog was removed from the bath and observed until he was able to breathe adequately without oxygen or an

Table 1

DOGS		EXPERIMENTAL PROCEDURE AND RESULTS	MIN. OF OCCLUSION	RECTAL TEMP. DURING OCCLUSION, °F.	AUTOPSIED P O DAYS	CLINICAL OBSERVATION
Control	I	No occlusion, hypothermia	—	78	Immed	—
	II	No occlusion, hypothermia & rewarmed	—	79	2 hours	—
	III	No procedure	—	99	Immed	Normal animal
Acute	XB0	Occlusion, cavae	10	77	4 hours	Did not arouse
	XB1	Occlusion, cavae	10	78	24 hours	Awake, eating
	XB2	Occlusion, cavae	10	77	48 hours	Alert, eating
	XB3	Occlusion, cavae	10	80	7 days	Normal dog
	XB4	Occlusion, cavae	10	77	72 hours	Normal dog
	XB5	Occlusion, cavae	10	82	5 days	Normal dog
	XB6	Occlusion, cavae	10	80	4 days	Normal dog
	XB7	Occlusion, cavae, died, ventricular fibrill	10	75	—	—
	XB8	No occlusion, ventricular fibrill	—	66	—	—
Chronic	XA1	Occlusion, cavae & aorta	8½	77	31 days	Vicious
	XA2	Occlusion, cavae & aorta	8	82	24	Normal
	XA3	Occlusion, cavae & aorta	10	79	31	Vicious
	XA4	Occlusion, cavae & aorta	12	76	31	Vicious
	XA5	Occlusion, cavae	10	77	31	Normal
	XA6	Occlusion, cavae, died, ventricular fibrill	10	78	—	—
	XA7	Occlusion, cavae	10	78	31	Normal
	XA8	Occlusion, cavae, died, ventricular fibrill	10	78	—	—
	XA9	Occlusion, cavae	10	79	10	Jacksonian seizures, right
	XA10	Occlusion, cavae	10	81	31	Vicious
	XA11	Occlusion, cavae	10	78	31	Unmanageable
	XA12	Occlusion, cavae, died 12 hr, ventricular fibrill	10	76	—	—
	XA13	Occlusion, cavae	10	79	22	Normal
	XA14	Occlusion, cavae; died, ventricular fibrill	10	77	—	—
	XA15	Occlusion, cavae	10	76	31	Normal
	XA16	Occlusion, cavae & aorta, died 3 hr, ventricular fibrill	10	77	—	—

endotracheal tube. He was then placed in a warm room for the next twelve hours.

CLINICAL OBSERVATION

There were seven deaths in this series of twenty-eight animals (Table 1). One animal, with a rectal temperature of 66°F., died of ventricular fibrillation before any occlusion. The other six deaths were from ventricular fibrillation; four during occlusion, one in three hours, and one in twelve hours. None of the deaths were due to nervous system damage, but were apparently due to a cardiac deficiency.

Of the remaining eighteen animals, all survived the operative procedure, with occlusion of eight and one-half to twelve minutes (Table 1). Most received occlusion of ten minutes. One animal, XA9, was sacrificed on the tenth postoperative day because of uncontrollable jacksonian seizures. Two other animals died of massive lobar pneumonia, one at twenty-two days and one at twenty-four days.

Animals which recovered from the operative procedure were very logy for the first twenty-four to forty-eight hours. Within twenty-four hours, all except XA9 were able to stand and drink milk; after forty-eight hours they were lively in their cages, and eating well. XA9 did poorly immediately postoperative. He stood with difficulty on the second postoperative day, and ate poorly. On the ninth postoperative day he was noted to have right-sided jacksonian seizures. The seizures became uncontrollable, and he was sacrificed on the tenth postoperative day.

Of the animals surviving thirty-one days, eight in number, five showed personality changes (Table 1). One was mean and unmanageable, and the other four were noted to be more vicious. All ate well. There were no abnormal neurologic findings. Fundoscopic examinations were not done. The personality changes were usually noted after the twenty-fourth postoperative day.

ELECTROENCEPHALOGRAM STUDIES

Electroencephalogram studies were carried out on ten dogs who survived the operative procedure. Two days prior to surgery extradural electrodes were placed through small skull slits over the frontal, parietal, and occipital regions. These electrodes were silver disks, 30 sq. mm., and connected to the outside by plastic-covered silver wires which were brought through the muscle in the occipital region. Observation of the electrodes at autopsy revealed them to be held firmly in place on the dura by fibrous reaction. No cortical reactions were noted under the electrodes.

Electroencephalograms (EEG) were taken preoperatively, during refrigeration, during occlusion, immediately after operation, after recovery of normal temperature, and at three, ten, seventeen, twenty-four, and thirty-one days. EEGs were taken on an eight-channel Grass machine, Model II, calibrated at 50 microvolts equal 7 mm., and run at 3 cm. per second.

Preoperatively, the EEGs were normal. There was little cortical activity with the temperature below 83°F. The cortical activity present was of reduced amplitude, slower, and with absence of 10 to 12 per second spindle formation. These observations were similar to those reported by Callaghan et al. in the monkey[12] (Table 2).

Table 2.

DOG NO	EEG PRE-OP	DURING EXPERIMENT: REFRIGERATED BEFORE OCCLUSION	SECS OF OCCLUSION TILL ALL ELECTRICAL ACTIVITY CONSIDERED ABSENT	ONE HOUR POST-OCCLUSION, STILL REFRIGERATED	TWO HOURS POST-OCCLUSION, NORMAL TEMP	FIRST POST-OPERATIVE WEEK	FINAL EEG PRIOR TO AUTOPSY	CLINICAL OBSERVATION
XA1	—	—	—	—	—	—	—	Vicious
XA2	Normal	Decreased voltage and marked slowing Absent 10-12 per sec spindles	30	No cortical activity	Low voltage, irregular, slow	Normal	Normal	Normal
XA3	Normal	ibid	40	ibid	No cortical activity	Abnormal, decreased amplitude in occipitals with occasional spikes	Abnormal, same as 1 week	Vicious
XA4	Normal	ibid	70	ibid	Slightly slower with decreased amplitude	Abnormal, left occipital—slower with decreased amplitude	Abnormal, generalized slowing with decreased amplitude in occipitals	Vicious
XA5	Normal	ibid	50	ibid	Slow with decreased amplitude	Normal	Normal	Normal
XA6	Normal	ibid	35	Died	—	—	—	—
XA7	Normal	ibid	70	No cortical activity	Mild slowing with decreased amplitude	Normal	Abnormal, amplitude *assymetry left fronto*-parietal	Normal

XA8	*Normal*	*ibid*	*60*	*Died*	—	—	—	—
XA9	Normal	ibid.	35	No cortical activity	Slow with decreased amplitude Worse in left parietal	Abnormal, generalized slowing, most marked in left parietal	Abnormal, generalized slowing with marked left parietal focus	Jacksonian seizures, right
XA10	Normal	ibid.	6	ibid.	Moderate slowing with decreased amplitude	Normal	Abnormal, amplitude assymetry left hemisphere	Vicious
XA11	Normal	Almost complete absence of cortical activity	20	ibid.	Mild slowing with decreased amplitude	Normal	Abnormal, mild slowing and decreased amplitude left parietal	Unmanageable
XA12	Normal	ibid.	15	No cortical activity	No cortical activity. Died 12 hours	—	—	—
XA13	Normal	ibid	20	No cortical activity	Marked slowing and decreased amplitude	Normal	Normal	Normal
XA14	Normal	Decreased voltage and marked slowing absent 10-12 sec. activity	65	Died	—	—	—	—
XA15	Normal	ibid.	—	No cortical activity	No cortical activity. Died 1 hour	Normal	Normal	Normal
XA16	Normal	Almost complete absence of cortical activity	30	*No cortical activity*	*No cortical activity.* Died 1 hour	—	—	—
			Average 39 sec.			10 LEGS 3 Abnormal 7 Normal	10 LEGS 6 Abnormal 4 Normal	11 ANIMALS 6 Abnormal 5 Normal

Six to seventy seconds following occlusion of the cavae, all cortical activity disappeared, the average time being thirty-nine seconds (Table 2). Forty-five to sixty minutes following the end of occlusion, and prior to re-warming, no cerebral activity had returned When the temperature had been restored to normal (two to three hours after occlusion), cortical activity was again present. In all cases, it was slower and of a much lower amplitude (Table 2).

Four animals had normal EEGs throughout the period of observation (Table 2). The rest of the animals developed EEG abnormality. One dog developed left parietal slowing in the thirty-one day record. Two had reduced amplitude over the left hemisphere. One had slowing and decreased amplitude with no focus. One, XA9, had slowing in the left parietal area on re-warming which became a left cerebral seizure focus by the time of his sacrifice on the tenth postoperative day. This correlated with the findings of right jacksonian seizures.

There were six abnormal EEGs (Table 2), a rate of sixty per cent. Five of the animals with EEG changes were noted to have neurologic or personality changes prior to sacrifice.

PATHOLOGY

Pathology studies were carried out on twenty-one animals. Controls used were: a normal animal; a normal animal during hypothermia; a normal animal immediately after hypothermia and re-warming Studies on dogs with occluded circulation were made at one, two, three, four, five, seven, ten, twenty-two, and thirty-one days. The thirty-one day group included eight dogs. Specimens were obtained after bleeding the dog, perfusing the head with saline, and then with 95 per cent alcohol. The brains were removed, and kept in absolute alcohol until sectioned.

Grossly, all the brains appeared normal, except XA9. On cut surface, no abnormality was noticed. There was no evidence of change under the EEG electrodes. The brain of XA9 was softer than normal, especially over the left parietal region. This was the only gross change

Sections were taken from the frontal, frontoparietal (motor and sensory area), occipital, and cerebellar regions Serial sections were not made. In the frontal and frontoparietal regions, basal ganglia and thalamic areas were included. With the cerebellar sections, pons and the floor of the fourth ventricle were included.

Kluver's stain for both cellular detail and myelin was used Sections from different animals were compared, area for area.

Histologically, it was found that the two experimental groups differed from the control group and from each other.

Group B showed evidence of neuronal damage. This consisted of chromatolysis and cell degeneration in the neurons of the cerebral cortex, generally, without accompanying inflammation or satellitosis. In the cerebellum, there was definite chromatolysis of the Purkinje cells. No definite changes in the myelin, basal ganglia, or brain stem were observed.

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Included in group A was XA9. There was an extensive area of subcortical

necrosis in the left frontoparietal region, characterized by many gitter cells. Except for this one animal the changes were minimal in each group. We were unable to demonstrate the damage of anoxia reported by other observers after occlusion of circulation.[14,15] There was no evidence of laminar degeneration or marked cellular degeneration-pyknosis, ghost cells, etc. Though of a minor nature, changes were definitely present. These changes were defined only by careful comparison with the control group and with each other. The brains of more animals will have to be examined before definite conclusions can be drawn, but our tentative conclusion is that a distinction can be made between acute and chronic changes on the basis of histologic observations alone.

CONCLUSION

From the evidence accumulated here, we can say that ten minutes of vascular occlusion under refrigeration anesthesia produces demonstrable change in the central nervous system.

From a clinical and electrophysiologic standpoint, there were significant changes in six of ten of the animals studied. The most severe of these changes was a jacksonian seizure focus. The other changes, while not as severe, were definitely present. In five of the animals, the clinical changes correlated with the electroencephalograph abnormality.

Pathologic changes in the central nervous system were of a minor nature. Acute and chronic changes were present and identifiable. Histologic differentiation of the various groups is possible.

Refrigeration anesthesia does protect the central nervous system to a great extent. Occlusion, without hypothermia, causes definite neurologic and pathologic changes in three and one-half to five minutes, and death or permanent coma in six minutes.[15] With refrigeration anesthesia, we are able to protect the brain. This protection is not absolute in the dog for ten minutes of occlusion, with the body temperature 76° to 82°F

SUMMARY

The results of a series of experiments on dogs, testing the efficacy of refrigeration anesthesia in protecting the central nervous system during complete vascular occlusion, are presented. Animals studied had temperatures of 76° to 82°F. Occlusion of the vascular tree was from 8½ to 12 minutes. Studies were carried out by clinical observation, electroencephalograms, and gross and microscopic examination of the brain Acute, chronic, and controls were studied.

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OBSERVATIONS ON PROLONGED HYPOTHERMIA IN THE DOG*

BERNARD FISHER, JOHN HAPPEL, CLEM RUSS, AND PAUL PRENDERGAST

Numerous detailed studies regarding hypothermia have been reported upon in the last few years. Practically all have been concerned with methods of cooling, reduction of temperature to lower levels than previously obtained, and the immediate metabolic effects of the cooling and rewarming process. Few observations upon the effects of prolonged hypothermia have been reported. The majority of these have been concerned with patients subjected to general hypothermia. Smith and Fay,[1-3] in 1939–1940, were the first to use hypothermia in the treatment of cancer. Its use in schizophrenic patients has been evaluated.[4] In the experimental animal, a few observations have been made, but these have chiefly been concerned with survival.[5-8]

In this laboratory it has been possible to maintain animals hypothermic for relatively long periods of time so that metabolic studies heretofore not reported could be made Our experiences with such a preparation in 12 dogs form the basis of this report of work in progress.

EXPERIMENTAL

Mongrel dogs of both sexes, averaging 10.3 kg. in weight, were anesthetized by open drop ether, except in one instance, where pentobarbital so-

* From the Department of Surgery, Section on Surgical Research and the Addison H. Gibson Laboratory, University of Pittsburgh This study was supported by Contract No. DA-49-007-MD-518, Department of the Army

dium intravenously was used (dog No. 803). Preliminary to cooling, polyethylene catheters were inserted via the femoral artery and vein into the aorta and inferior vena cava. An endotracheal tube with inflatable cuff was placed in the trachea, and a catheter was passed into the urinary bladder. Body temperature was measured by a thermometer inserted well into the rectosigmoid. Electrocardiographic leads were attached to the animal. Base line determinations were obtained. These included temperature, pulse, EKG tracing, respiration, arterial and venous pressure, and blood samples for CO_2, electrolytes, sugar, pH, specific gravity, prothrombin, Lee-White clotting time, urinalysis, and hematocrit. Conventional procedures were resorted to in these determinations. In several instances cardiac outputs using the Fick method, hepatic blood flow by the Bromsulphalein method of Bradley, and renal blood flows wi
formed. This necessitated
the right external jugular

Table 1.

EXP. NO.	DOG NO.	WEIGHT DOG (KG.)	LENGTH HYPO. (HRS.)	TIME IN BATH (MIN.)	30°-25°C. TIME (MIN.)	REMARKS
1	720	12.5	18	12	117	Died 2½ hrs. after rewarming
2	745	6.6	24	18	117	Died 6½ hrs. after rewarming
3	751	13.6	9	22	45	Died, cardiac arrest
4	761	10.3	24	25	88	Survived rewarming
5	784	8.2	24	12	178	Died during rewarming
6	787	8.0	15	15	89	Died, ventricular fibrillation
7	789	7.2	20	16	76	Died 4 hrs. after rewarming
8	790	7.1	24	16	45	Died 12 hrs. after rewarming
9	803	12.0	20	35	7	Died 4 hrs. after rewarming
10	805	10.6	24.5	29	79	Died 3 hrs. after rewarming
11	850	13.8	28	17	30	Died, respiratory arrest followed by ventricular fibrillation
12	868	14.7	35	29	45	Died, cardiac arrest
Avg.		10.3	24.6	20.5	81	

other deep into a hepatic vein. Approximately one hour was necessary for this preliminary preparation. Animals still under light ether anesthesia were placed in a water bath thermostatically controlled at 1°C. When the rectal temperature was 29° to 30°C. they were removed from the bath and placed in an air conditioned room for the duration of the experiment. By turning the cooling system off and on, the temperature of the room could be adjusted. Observations previously indicated were made at intervals until the termination of the experiment. Animals were rewarmed by placing them in a warm water bath of 30° to 36°C. until body temperatures reached the precooling level. In most instances the course of the animals was not interfered with by attempting to correct the physiologic changes encountered. In some, however, an attempt was made to reverse the more obvious alterations. Glucose, for example, was administered to correct the severe hypoglycemia, and fluids which seemed in order were given to note their effect on the progressive hemoconcentration that occurred.

RESULTS

As may be seen from Table 1, the average length of time of hypothermia (body temperature below normal) was 24.6 hours (with a range of from 9 to 48 hours). In order to accomplish this, animals were in the 1°C. bath for an average time of 20.5 minutes (12 to 35 minutes), during which time the body temperature was reduced to 30°C. Following removal from the bath, it took approximately 1½ hours for the animal to lower its temperature to

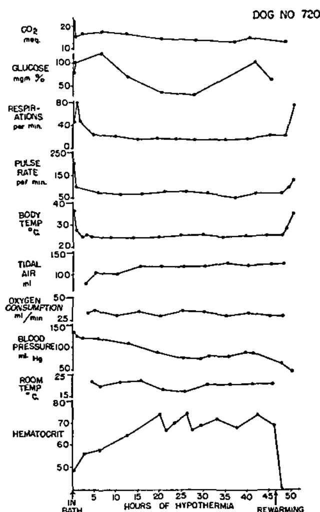


Fig 1. Observations during 48 hours of hypothermia.

25°C. Frequently more ether had to be administered to quiet the animals during this period. In all animals there seemed to be a critical temperature at which no more anesthesia needed to be administered. In most dogs this was at or slightly below 24°C. It was here that it was felt by us that the true effects of hypothermia were being encountered. Above this temperature, anesthesia, rather than cold itself, played a dominant role in depressing the animal. It has not been uncommon to observe animals between 24° and

25°C. raise their heads from the table practically fully awake. It was for this reason that ether, which was rapidly blown off, rather than pentobarbital sodium, was used.

Some of the specific effects produced by the cooling in this group of animals may be best summarized by presenting the findings in two representative dogs, No. 868 and No. 720, which were followed under hypothermia for 35 and 48 hours, respectively (Figs. 1 and 2).

After an initial period of stabilization, the temperature varied no more than 1°C. until rewarming. Pulse, respiration and oxygen consumption were likewise consistent. It is of interest to note that the tidal air is practically that of a normal dog, even at temperatures of 23°C. after many hours of cooling. This is not the picture with Nembutal anesthesia, where artificial respiration must be resorted to. The rise in blood sugar seen at the 28 hour mark is due to the administration of glucose when the blood sugar reached

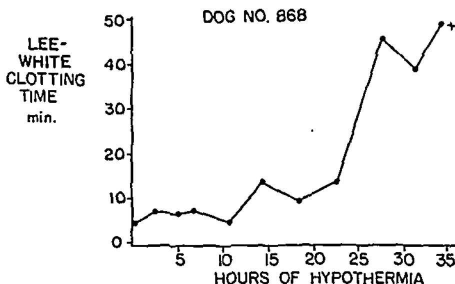


Fig. 2. Changes in Lee-White clotting time during prolonged hypothermia.

a level of 28 mg. per cent. The initial rise in blood sugar concentration and the fall in CO_2 may be attributed to ether anesthetic. Control experiments tend to substantiate this. The blood pressure tends to fall in the first hour of the experiment and then levels off at about 60 mm. Hg until just before death, when it falls to extremely low levels (20 to 30 mm. Hg). In 20 hours the hematocrit rose from 49 to 73, and then, with the administration of fluid (5 per cent glucose and normal saline), there were slight lowerings of hematocrit, but not until rewarming was there a return to the normal level.

The Lee-White clotting time, as seen in dog No. 868, shows interesting changes (Fig. 2). In the first few hours of cooling there is a slight progressive elevation of clotting time. At about 20 hours, there is a precipitous breakdown of clotting so that marked prolongation occurs (50 minutes at 30 hours of hypothermia). Preliminary observations on prothrombin time tend to parallel this. Cardiac outputs were done on dog No. 868 and were found to be as follows:

LENGTH OF HYPOTHERMIA (HOURS)	DOG TEMP. °C.	CARDIAC OUTPUT ML./MIN.	CARDIAC INDEX
2	24	1024	1.52
14	24	382	.57
28	23	269	.40

CONCLUSIONS

It is possible to maintain animals at low temperatures long enough (as long as 48 hours) to make pertinent metabolic observations. However, in our hands there has been little success in rewarming with survival of these animals. Data of interest reported here are the breakdown of the clotting mechanism, the marked decrease in cardiac output, and the effects on other vital functions that occur.

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EFFECT OF DRUG INDUCED HYPOTENSION ON THE CEREBRAL CIRCULATION IN MAN*

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Hypotension appears to be a useful adjunct in the control of operative hemorrhage. For years surgeons have taken advantage of the decrease in bleeding that occurs during hemorrhagic shock. Some have purposely produced hypotension by blood-letting. Since 1952, hypotension during surgery has been created deliberately by means of ganglionic blocking drugs, the most popular being hexamethonium bromide.[1] More recently a short acting sympatholytic drug, Arfonad,† has been introduced[2] This drug has the advantage of being more controllable than hexamethonium. Although hypo-

* From the Departments of Anesthesiology, Gastroenterology, Pharmacology and Physiology, Graduate School of Medicine, University of Pennsylvania and from the Harrison Department of Surgical Research and the Hospital of the Graduate School of Medicine, University of Pennsylvania, Philadelphia This study was aided by grants from the Wilson C. Laucks Memorial Foundation, the A. Atwater Kent Fund, and No. H-1282 from the National Heart Institute of the National Institutes of Health, Public Health Service

† Arfonad is *d* 3,4(1', 3'-dibenzyl-2'-keto-imidazolido)-1,2-trimethylene thiophanium *d*-camphor sulfonate, or RO2-2222. It was supplied by Hoffmann-LaRoche, Nutley, N. J.

tension exerts profound effects upon the circulation to vital organs, it has been intentionally used many times with relatively few serious consequences. The purpose of this study was to determine the effects of these drugs on the cerebral circulation and metabolism of individuals typical of those usually selected for hypotensive anesthesia.

METHOD OF STUDY

The subjects studied were hospital patients without evidence of vascular disease. In 10 patients hypotension was induced by giving intermittent doses of hexamethonium bromide intravenously. Usually this technique was used with the patient supine, at times supplemented by head-up tilt of the table. Given as an intravenous drip, Arfonad produced hypotension in three other patients. All 13 patients were studied in the same manner.

After a 30 minute stabilization period a cerebral blood flow determination was performed and calculated according to the original nitrous oxide method.[3] Hypotension was then induced by administering either hexamethonium or Arfonad, during which time a second blood flow determination was performed. Samples of arterial and internal jugular blood were analyzed for oxygen and carbon dioxide values using the manometric methods of Van Slyke.[4] They were also analyzed for pH anaerobically in a glass electrode, corrected for temperature by suitable factors.[5] Arterial and internal jugular blood pressures were measured directly by strain gauge and water manometers, respectively. Mean pressure was calculated by planimetry.

RESULTS OF ADMINISTRATION OF HEXAMETHONIUM

Of the ten patients receiving hexamethonium (Tables 1 and 2), six had asymptomatic hypertension (mean arterial blood pressures over 105 mm. [illegible] for surgery. With a fall in mean arterial blood pressure from 146 to 78 mg. Hg (47 per cent fall), cerebral blood flow did not change significantly owing to the decrease in cerebral vascular resistance from 2.9 to 1.6 resistance units (millimeters Hg per cubic centimeter of blood per 100 grams of brain per minute). Novack and Shenkin[6] have shown that, in individuals with asymptomatic, labile hypertension, cerebral vascular resistance can be reduced from 2 6 to 1.7 resistance units by carbon dioxide inhalation.

In the remaining three patients receiving hexamethonium, normotensive blood pressures fell from 96 to 58 mm. Hg (37 per cent fall) with no significant change in cerebral blood flow. Cerebral vascular resistance fell from 1.7 to 1.1 resistance units to maintain blood flow. Cerebral oxygen consumption was unaltered in both groups of patients. Statistical analysis of hypertensive and normotensive groups, as a whole, varied little from the study of each group separately. It can be assumed that the cerebral circulation of the patient with labile, asymptomatic hypertension reacts to induced hypotension at least as well as the normal does.

Blood gases were studied on the combined groups receiving hexamethonium as a whole (Table 2). Oxygen content fell uniformly in both arterial and internal jugular blood with no significant change in (A-V) oxygen difference. This is reflected in the sustained cerebral oxygen consumption. In Morris' study[7] of a group of patients receiving hexamethonium, there

Table 1. Effects of Drug Induced Hypotension on Cerebral Hemodynamics

PATIENT	AGE	SEX	MEAN ARTERIAL BLOOD PRESSURE (MM /HG)		CEREBRAL BLOOD FLOW (CC /100 GM /MIN.)		CEREBRAL VASCULAR RESISTANCE (MM HG/CC./ 100 GM /MIN)		CEREBRAL OXYGEN CONSUMPTION (CC O_2/100 GM / MIN)		TOTAL DOSE (MG)
HEXAMETHONIUM-HYPERTENSION											
			C	H	C	H	C	H	C	H	
1. D N	42	F	154	96	51	54	3 7	3 8	3 7	3 8	225
2 H R	61	F	130	36	41	37	3 0	0 8	2 2	2 0	50
3 E.C	40	F	143	80	45	48	3 0	1 7	2 9	4 3	75
4 M Y *	43	F	162	68	84	50	1 7	1 1	5 2	5 1	100
5 N R *	49	M	113	98	36	43	3 8	2 1	1 5	2 7	175
6 E B *	43	F	145	92	38	33	3 4	2 3	3 5	2 9	75
Mean	47		146	78†	49	44	2 9	1 6‡	3 2	3 5	
S E			±4 9	±11	±7 3	±1 0	±0 29	±0 25	±0 53	±0 45	
HEXAMETHONIUM-NORMOTENSION											
7. F B	29	F	102	59	47	19	2 1	1 2	3 0	2 5	125
8 L M	26	F	99	79	94	67	0 9	1 0	5 0	3 7	87
9 A S *	60	F	99	44	46	49	2 2	0 9	3 0	4 1	150
10 J D *	33	M	83	49	48	43	1 7	1 0	2 8	3 3	150
Mean	37		96	58‡	59	52	1 7	1 1‡	3 4	3 4	
S E			±4 4	±7 7	±12	±5	±0 3	±0 2	±0 6	±0 3	
ENTIRE HEXAMETHONIUM GROUP											
Mean	43		126	70†	53	47	2 4	1 3†	3 3	3 4	
S E			±9 1	±7 6	±6 2	±2 0	±0 3	±0 2	±0 4	±0 3	
ARFONAD											
1 J S	53	M	79	47	31	28	2 6	1 7	—	2 3	500
2 G H	30	M	84	28	30	28	2 6	0 8	2 5	—	425
3 C C.	43	M	96	44	38	39	2 3	0 8	2.8	3 8	475
Mean	42		84	39	33	32	2 5	1 1	2.7	3 0	

C = Control H = Hypotension *10° head-up tilt †p<0 01. ‡p<0 05

Table 2. Effects of Drug Induced Hypotension on Blood Values

Patient	Arterial O_2 Content (vol. %) C	Arterial O_2 Content (vol. %) H	Venous O_2 Content (vol. %) C	Venous O_2 Content (vol. %) H	A-V O_2 Difference (vol. %) C	A-V O_2 Difference (vol. %) H	O_2 Capacity (vol. %) C	O_2 Capacity (vol. %) H	Hematocrit C	Hematocrit H	Arterial CO_2 Content (vol. %) C	Arterial CO_2 Content (vol. %) H	Venous CO_2 Content (vol. %) C	Venous CO_2 Content (vol. %) H	A-V CO_2 Difference (vol. %) C	A-V CO_2 Difference (vol. %) H	Arterial pCO_2 (mm Hg) C	Arterial pCO_2 (mm Hg) H	Venous pCO_2 (mm Hg) C	Venous pCO_2 (mm Hg) H	Arterial pH C	Arterial pH H	Venous pH C	Venous pH H	Venous Pressure (mm Hg) C	Venous Pressure (mm Hg) H
Hexamethonium induced hypotension																										
1 DN	16.6	15.5	9.3	8.4	7.3	7.1	17.3	—	—	—	41.2	40.5	48.5	48.3	7.3	7.8	34	32	42	41	7.40	7.42	7.37	7.35	15	15
2 HR	15.6	13.2	10.1	7.7	5.5	5.5	17.0	—	—	—	51.8	51.3	56.5	56.9	4.7	5.6	45	45	54	55	7.38	7.38	7.33	7.33	6	5
3 EC	15.7	15.1	9.3	6.2	6.4	8.9	17.7	—	—	—	54.0	54.8	61.1	62.3	7.1	7.5	45	45	54	54	7.40	7.41	7.37	7.37	7	0
4 MY	16.5	15.5	10.3	5.2	6.2	10.3	17.6	—	—	—	54.5	55.2	60.3	64.6	5.8	9.4	36	40	53	52	7.44	7.47	7.36	7.41	21	15
5 NR	13.7	13.8	9.6	7.5	4.1	6.3	16.1	—	—	—	49.4	48.9	52.9	56.0	3.5	7.1	42	46	49	56	7.38	7.31	7.34	7.30	5	10
6 EB	16.9	14.6	7.8	5.9	9.1	8.7	17.1	—	—	—	42.0	45.6	51.3	53.7	9.3	8.1	32	35	42	46	7.44	7.45	7.40	7.38	17	16
7 FB	16.4	15.0	10.1	9.9	6.3	5.1	16.8	—	—	—	47.5	—	52.9	—	5.4	—	—	—	—	—	—	—	—	—	4	0
8 LM	15.6	15.1	10.3	9.6	5.3	5.5	15.7	—	—	—	46.4	48.9	52.8	51.2	6.4	5.3	—	—	—	—	—	—	—	—	14	13
9 AS	14.8	13.7	8.3	5.4	6.5	8.3	15.4	—	—	—	52.0	51.8	58.5	59.0	6.5	7.0	44	43	54	51	7.38	7.39	7.34	7.33	6	8
10 JD	16.2	16.1	10.5	8.3	5.7	7.8	18.1	—	—	—	54.7	52.7	58.8	61.0	4.1	8.3	42	41	48	52	7.44	7.43	7.41	7.38	4	5
Mean	15.8	14.7*	9.5	7.4	6.3	7.3	16.9				49.4	50.0	55.4	57.3*	6.0	7.4	40	41	50	52	7.41	7.41	7.37	7.36		
SE	±0.3	±0.3	±0.3	±0.5	±0.4	±0.5	±0.3				±1.6	±1.5	±1.3	±1.6	±0.6	±0.4	±2	±2	±2	±2	±0.01	±0.02	±0.01	±0.01		
Arfonad induced hypotension																										
1 JS	15.0	15.4	—	6.9	—	8.5	15.7	15.3	—	—	49.8	48.1	—	57.1	—	9.0	39	37	—	48	7.39	7.44	7.35	7.38	0	0
2. GH	18.6	21.2	10.3	—	8.3	—	18.5	23.2	47	52	43.9	42.2	50.2	—	6.3	—	37	37	47	—	7.35	7.36	7.32	7.34	7	5
3 CC	17.5	15.7	10.2	6.0	7.3	9.7	17.5	17.0	43	44	49.5	48.4	56.9	58.0	7.4	9.6	40	36	50	50	7.42	7.42	7.37	7.37	8	10
Mean	17.0	17.7	10.3	6.5	7.8	9.1	17.2	18.5	45	48	47.8	46.2	53.6	57.6	6.9	9.3	39	37	49	49	7.39	7.41	7.35	7.37		

C = Control H = During hypotension
*$p < 0.01$

was a significant increase in plasma volume as measured by radioactive iodinated albumin. *With hypotension an average plasma volume increase of* 17 per cent occurred. In as yet unpublished studies of other hypotensive methods, we found that a similar fall in oxygen content accompanied by a proportional decrease in oxygen capacity occurred. It became apparent that the fall in oxygen content is a manifestation of hemodilution and not of decreased oxygen saturation, occurring as a result of either hypotension or relative blood volume discrepancy, or both. Studies of the other blood components showed no significant changes, with the exception of the internal jugular carbon dioxide content. The reason for this single change is not clear.

RESULTS OF ADMINISTRATION OF ARFONAD

Despite a fall in mean arterial blood pressure from 84 to 39 mm. Hg (52 per cent) after Arfonad (Table 1), there was no appreciable change in cerebral blood flow. A decrease of cerebral vascular resistance from 2.5 to 1.1 resistance units accounted for maintenance of blood flow at control levels. Cerebral oxygen consumption showed little change.

In contrast to the series receiving hexamethonium the group receiving Arfonad showed evidence of hemoconcentration. Oxygen content and capacity as well as hematocrit values increased. However, oxygen saturation fell in both arterial and internal jugular blood. The effect of Arfonad on the pulmonary circulation may prove to be an important factor as witnessed by the form of the arterial nitrous oxide uptake curves. During Arfonad hypotension the arterial curve approached stabilization more slowly than during the control study. The significance of this trend may be clarified when further studies are made

As with the hexamethonium series, this group showed a sizable rise in internal jugular carbon dioxide content while all other blood values changed very little.

DISCUSSION

What is the mechanism of the decreased cerebral vascular resistance during hexamethonium and Arfonad induced hypotension? Vascular resist- [illegible] produced by a [illegible] gocornine,[9] pro- [illegible] anesthesia.[13] It decreases also in hypotension in which drugs have not been used. An excellent example of this occurs in early induced hemorrhagic shock, where it decreases when mean arterial blood pressure falls.[14] It is reasonable to assume that cerebral vascular resistance decreases as a result of hypotension itself, whatever the cause This is especially true in this study since blood metabolites could not be implicated and since direct effect of these drugs on cerebral vessels has never been demonstrated. Vascular resistance was considered to be due primarily to vessel tone since other factors varied so little.

The results of this study differ with those of Moyer and co-workers,[15] who used the same drugs. They found significant fall in cerebral blood flow with both hexamethonium and Arfonad. This might be explained by the fact that mean arterial blood pressure falls (hexamethonium, 37 per cent fall; Arfonad, 42 per cent fall) obtained by them were less than presented in this

study. Thus the intensity of the stimulus to cerebral vascular dilatation w less. In addition, experimental cerebral blood flow levels that they report were still compatible with cerebral oxygenation.

An interesting sidelight with bearing on the picture of hypotension a cerebral blood flow was noticed during the Arfonad study. An instance contamination of internal jugular blood by blood of extracerebral orig occurred during the control blood flow. The criteria of contamination we those of Kety,[3] emphasized by Sokoloff.[10] During Arfonad hypotension, ho ever, evidences of contamination were absent, without any change in t location of the internal jugular needle or the procedure. This is addition evidence for the shunting of blood from somatic tissue to vital organs su as the brain, during periods of low blood pressure. This shift from co tamination was also observed in the analysis of several of the patier studied during hexamethonium hypotension and hemorrhagic shock. these cases contamination was never considered to be significant.

SUMMARY

1. Thirteen patients were studied during intentional hypotension wi either hexamethonium bromide or Arfonad.

2. In both groups a significant fall in blood pressure produced litt change in cerebral blood flow.

3. During hexamethonium induced hypotension there was evidence hemodilution, while following Arfonad the trend was toward hemoco centration.

4. Arterial oxygen saturation was decreased in the group receivi Arfonad.

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A QUANTITATIVE EXPERIMENTAL COMPARISON OF THE EFFECTS OF SEVERE HYPERCAPNEA ON THE BRAIN AND HEART*

A. L. HOPKINS, JORGE ANZOLA, AND GEORGE H. A. CLOWES, JR.

A major respirat
This makes it im
whole organism.
brain is profoundly affected and damaged by excess carbon dioxide

To date, the effects of CO_2 on the heart have received the most attention, and it has become apparent that this organ is relatively insensitive As early as 1928 Schmidt[1] was able to maintain dogs for 15 minutes on 80 per cent CO_2. Recently Miller and Brown[2, 3] have studied the effect of CO_2 on the cardiograms of man and dogs, in dogs they found very little change with mixtures up to 60 per cent CO_2 and were able to go up to 90 per cent before death occurred.

The experiments to be reported concern the general effects of elevated partial pressures of CO_2 in dogs. A previous report[4] showed that the electroencephalographic depression caused by anesthesia is potentiated by the presence of an elevated arterial pCO_2. That this depression is of considerable clinical significance is suggested by the delayed death of a majority of the animals in which the brain waves were absent for fifteen minutes or more.

METHODS AND MATERIALS

Mongrel dogs weighing between 20 and 30 pounds were used. To assure a constant insufflation of the lungs with the desired gas mixture, a positive

* ...stern Reserve University School of Medicine at ...stigation was supported in part by research grant ... National Institutes of Health, Public Health Service.

pressure endotracheal respirator was used. The rate was constant at 25 respirations per minute while the tidal volume could be varied. Electroencephalographic and electrocardiographic recordings were made with a Grass ink-writing oscillograph Model III B. Arterial blood pressure was measured with a mercury manometer. Gas analysis of blood samples was done by the Van Slyke method. At the high CO_2 levels where the Singer and Hastings[5] nomogram could not be applied in the derivation of pCO_2, the data of Van Slyke[6] were used to estimate the pCO_2. The error involved in such estimations was found to be about 15 per cent when applied to data obtained from other blood samples in which CO_2 tensions had been measured by the bubble technique of Riley.[7] The pH was determined with a Cambridge pH meter; plasma K, with the Bird Associates flame photometer.

PROCEDURE

Normal dogs were anesthetized with Nembutal (60 mg. per kilogram) and connected to the respirator with an endotracheal tube. After being pumped on room air for 10 minutes, the initial blood samples were taken and the encephalograph was turned on. The respirator was switched from room air to the CO_2 mixture, which was continued for 20 minutes. During the exposure to CO_2, continuous records were made of the E.E.G. and E.C.G.; blood pressure was recorded every minute or 30 seconds. Three samples of arterial blood were taken at roughly five minute intervals for gas analysis. Five or six arterial blood samples were taken for determination of plasma K. At the end of 20 minutes the CO_2 was diluted gradually to avoid fibrillation. A total of 10 dogs were employed in these experiments, five of which were given 55 per cent CO_2.

RESULTS

As shown in Figure 1, the administration of 55 per cent CO_2 in O_2 produces a pH below 6.5 and a pCO_2 of about 400 mm. Hg. The oxygen content remains at or above 20 V. per cent. Usually within the first minute of exposure to CO_2 there is a rapid fall in mean arterial blood pressure and heart rate. Within two to four minutes both return to or exceed their original values. After five to six minutes of exposure, both slowly decline and usually continue to do so for 15 minutes. At the end of this time the rate of fall becomes very slight as a steady state is approached. Although the changes in blood pressure, heart rate, and E.C.G. are not striking at the end of 20 minutes of 55 per cent CO_2, a very profound change has taken place in the E.E.G. (Fig. 2): it has been completely flattened to the point where only background interference is being recorded. This flattening was preceded by a period of stimulation as evidenced by the E.E.G. temporary increase in wave frequency, which occurred simultaneously with the increase in blood pressure and heart rate. Typically the brain waves disappear from six to ten minutes after the animal has been switched from room air to CO_2. Even though the brain waves appear to be quite normal after the CO_2 has been removed and the animal in general seems to be in good condition, the mortality rate in the following 12 hours is rather high. The majority of the animals whose brain waves were absent for 15 minutes or more died.

In general, this sequence of
exposed to both higher and lo
above. The time required for t

to the strengths of CO_2 used, with 60 per cent the brain waves disappear sooner than with 40 per cent CO_2.

The plasma potassium showed an increase of 1 to 1.5 mEq. per liter over the control values, usually within the first five minutes. Also it has been noticed that serious arrhythmias, extrasystoles, and sometimes ventricular fibrillation occur if the animal is switched to room air from CO_2 without gradual dilution. Death does not occur during the experiment if the mixture

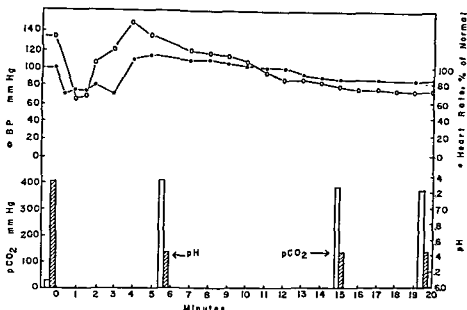


Fig 1. Mean arterial blood pressure (open circles), heart rate in per cent of normal (closed circles), pH, and calculated pCO_2 changes that occur in a typical experiment involving the administration of 55 per cent CO_2 which was begun at 0 minutes.

Before CO2 55% CO2 For 20 Min. After CO2, 10 Min

E.E.G.

E.C.G.

electrocardiographic
CO2, and 10 minutes

given does not exceed 60 per cent and the time of exposure does not exceed 20 minutes. Dogs died on CO_2 only when these limits were exceeded.

DISCUSSION

It is felt that in order to obtain consistent experimental results, the experimental conditions must be rigorously controlled and measured. Of particular importance is some device to insure that the animal receives the gas mixture at a constant rate throughout the experiment. By this method one eliminates the overbreathing that takes place during the initial stimulatory phase and the possibility of hypoxia resulting from the depressant action of

CO_2 that occurs in the later stages of the experiment. Though positive pressure respiration has the disadvantage of slightly inhibiting the venous return to the heart and of increasing the oxygen content of the blood, it is felt to be preferable to uncontrolled degrees of hypoxia.

These experiments demonstrate quite clearly that in dogs there is a very marked difference between the action of CO_2 on the brain and on the heart. Arterial CO_2 tensions which produce only slight changes in the electrocardiogram are sufficient to eliminate completely those brain waves which can be recorded from electrodes placed on the skull. It is felt that this difference in action on the heart and brain is of both experimental and clinical significance.

Similar brain wave depression accompanied by minimal electrocardiographic changes in the presence of an elevated pCO_2 have been recorded in man.[4] It is possible that the unexpected delayed death which occasionally occurs following prolonged and difficult surgery may be related to the observed mortality among dogs in which the brain waves were absent for periods of 15 minutes or more. It is suggested that irreversible damage and cellular death may occur at those levels of hypercapnea which are capable of completely eliminating the recordable electropotentials of the brain.

SUMMARY

Arterial pCO_2 levels that produce only mild changes in the electrocardiograms of dogs are sufficient to eliminate all brain wave potentials. Those animals whose brain waves have been depressed in this way have a high subsequent mortality rate. The significance of this in prolonged surgical procedures that involves CO_2 retention is suggested.

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BURNS

INTRODUCTION

JOHN M. HOWARD

With the increasing knowledge of the basic nature of thermal injuries and of the body's response to these injuries has come an increasing urgency for additional study. Military weapons on both sides of the world have now been developed to such a point that thermal injuries, like mechanical injuries, have assumed a tremendous importance. Investigation is thus developing in two spheres of interest: first, in understanding the injury, the response, and therefore the treatment of the individual patient; and secondly, *in how to apply this knowledge in the most practical manner to mass* casualties.

In understanding the burn injury, Hershey of Washington University, St. Louis, has approached the study of the wound at the very basic level of studying cellular function as measured by enzymatic activity. Such a continuing, long-range program as Moyer has underway will continue to aid in depicting the basic nature of the injury. While this type of study has been delineating the changes at the point of injury, other studies have been describing the effect of the local wound on the rest of the body. Payne and Krauel of the State University of Washington, Seattle, have taken one facet of the metabolic effect of the local wound, the lymphatic absorption of lipids *from the wound, and demonstrated beautifully one method in which* the local wound produces systemic effects. Finally, other investigations have demonstrated the responses of the body to the wound, responses and effects which may decide the life or death of the "uninjured portion of the body." Thus a 25 per cent third degree burn represents an injury in which 25 per cent of the body surface will die. But the wound is not an organism unto itself; the thermal injury affects not only the local site of injury, but the entire body. *The entire body is injured and the entire body responds to* fight for the survival of the "75 per cent," and to halt the insult and to repair

battle casualties,

body not for the

Only by understanding the nature of the original and the continuing elements of injury can one rationally approach the problems of therapy.

From the laboratories of the late Dr. Everett Evans in Richmond, and of the Army's Surgical Research Unit at Fort Sam Houston, continues to come valuable information regarding the care of burned patients. This work has been centered around the problems encountered in the care of mass casualties with thermal injuries. From the former laboratory Martin has analyzed their successful experience in resuscitating burned patients with dextran. It is essential to continue to gain information regarding the usefulness and limitations of these preparations which can be stock-piled in advance. As a result of such studies, compromises, necessary in *the treatment* of mass

casualties, can better be planned. Military conditions might suddenly confront us with 25,000 to 100,000 centralized burned casualties. It is with a realization of the need for rational planning for such potential problems that investigation and teaching in the fields of burns and trauma are being approached.

THE SUCCESSFUL USE OF DEXTRAN IN THE TREATMENT AND PREVENTION OF SHOCK IN THE BURNED PATIENT*

EVERETT I. EVANS† AND MARY M. MARTIN

Dextran has been used in the prevention and treatment of shock from burns in 123 patients. These patients were those requiring intravenous therapy in a group of 500 patients seen consecutively over a 24 month period. Their ages ranged from six days old to 90 years old. The extent of the burns varied from 10 per cent to 95 per cent of the body surface. The Swedish, British, American, and South African dextrans were used without preference. All dextran used was 6 per cent incorporated in normal saline.

Each patient requiring intravenous therapy (123 patients of 505 burns seen in the same period of time) was treated by immediate cut-down or by use of the Rochester plastic needle. Fluid was started as soon as cut-down was established. The first fluid given was always dextran, except that a few patients were given plasma at another hospital before arriving in the unit in Richmond. An accurate weight was ascertained. The exact percentage of body surface burned was mapped out for the specific age according to the figures of Lund and Browder.[1] A self-retaining catheter was inserted and the hourly urine output carefully recorded after the bladder was emptied. Occasionally, in the very young infant burned about the genitalia, maintaining an hourly urine collection proved impractical. The exact amount of colloid required to replace the loss in fluid for the 48 hours immediately following burning was calculated. The guide used as developed by Evans[2,3] was 1.5 cc. of colloid per kilogram of body weight per percentage point of body surface burned for the post-burn period of 48 hours. One cubic centimeter of colloid per kilogram per percentage point of burn was given the first 24 hours, and 0.5 cc. of colloid per kilogram per percentage point of [illegible] of sal [illegible] of wa [illegible] ce in [illegible] ly.

In third degree burns, or large second degree burns, half of the colloid requirement was given as whole blood. Blood was usually given to any patient received late after the initial injury and to those sustaining large second degree burns or third degree burns of moderate to large extent. It was necessary especially in small infants with even small third degree burns to give more than half of the amount of colloid required as whole blood. This may have been due in part to the large number of infants with a preexisting anemia who were burned.

No untoward reaction was observed during or after the administration of any of the dextran products used. There were no instances of urticaria or of anaphylactic reaction during administration.

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† Deceased.

The urine output was kept between 25 and 50 cc. per hour in the adult and correspondingly lower in the child. The urine output was found adequate the first 48 hours in all instances with the exception of patients dying from irreversi ... ie unit from 5 to 8 hours . ... n a narcotic before being ... ore the time of institution of shock therapy. The urine obtained from these patients on initial catheterization showed marked hemoglobinuria.

Fifty-four patients were treated with dextran alone. Sixty-six patients were treated with dextran and whole blood Six patients were treated with plasma, whole blood, and dextran. Three hundred and eighty 500 cc. bottles of dextran were used.

Analysis of the amounts of colloid actually administered to each of the patients and their courses has been made. This includes calculation of the actual amount administered per kilogram of body weight per percentage point of burn for the 48 hour post-burn period as compared with the theoretical amount necessary over the 48 hour period. Discrepancy was noted between the calculated amount required by formula and the actual amount administered in the patient with a small burn needing little intravenous fluid after the first 24 hours post burn

SUMMARY

Dextran is felt to be an adequate plasma expander in the resuscitation of the burned patient. It may be administered successfully according to the formula developed by Evans[2] Its effectiveness may be followed by measurement of the hourly urine output. It appears to maintain osmotic pressure as efficiently as, or more efficiently than, plasma as measured by adequate renal outflow. Instances in which its use alone is inadequate are those in which anemia is established prior to burning in the small burn or in extensive second and third degree burns requiring one-half or more of the calculated colloid to be given as whole blood.

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QUANTITATIVE HISTOCHEMISTRY OF BURNED AND NORMAL SKIN*

FALLS B. HERSHEY AND BARRY JILL MENDLE

The epidermis is the site of three phenomena of great surgical interest: the healing of a wound, the development of cancer, and local damage from burns.

BIOCHEMICAL APPROACH TO BURNS

Thermal injury must be attended by alterations in the metabolism of the epidermis. However, practically nothing is known about the fundamental metabolic processes in skin. Since enzymes have been shown to be most important elements for the maintenance of cellular life, the quantitative study of enzymes in the skin of man should be a logical approach. The purposes of this study were: (1) the diagnosis of the "biochemical lesion" in thermal burns; (2) the collection of data about metabolic enzymes of skin which might be helpful in skin preservation or banking and the development of therapy to reverse the chemical changes leading to delayed death of cells after their injury by heat; and (3) the collection of data as a background for the study of the enzymatic changes attending cancer and the healing of burns and other wounds.

METHODS

A scald of the skin of the back was produced with water of 50° C. applied for five minutes. One-half the scalds induced in this manner progress to complete transepidermal necrosis.[1] The skin and an adjacent normal sample were removed one-half to one hour after injury without anesthesia.

The methods employed in these analyses were designed and perfected by Dr. O. H. Lowry and his associates.[2-5] The tissue to be analyzed is frozen in liquid nitrogen, sectioned and dried under vacuum while still frozen. This preserves unstable enzymes. Selected pieces weighing approximately 1/1000 of a milligram are cut from the large sample under the dissecting microscope and accurately weighed on a quartz fiber balance.

RESULTS

Using these techniques it is possible to clearly separate and analyze three distinct layers of the skin, namely the keratin, cellular epidermis and dermis (Fig. 1). The results of the analyses of these three layers, taken from the sole of amputated extremities, are shown in Table I. Histochemical methods are available for only two of these, the acid and alkaline phosphatases. There are no practicable staining methods for the others, which are enzymes of three different systems for oxidation of carbohydrate. They have not been previously demonstrated in the epidermis of man.

Significant activities of malic and lactic dehydrogenase, acid phosphatase, and purine nucleoside phosphorylase are present in the keratin layer.

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In addition to the above, 6-phospho-gluconic dehydrogenase, aldolase, and fumarase are more highly active in epidermal cells.

Dermis possesses practically no purine nucleoside phosphorylase activity, but there is significant alkaline phosphatase activity.

All the enzymic activities in epidermal cells exceed those in the keratin layer with the exception of purine nucleoside phosphorylase. Similarly, the

Fig 1.



Fig 2

Fig. 1 Human sole, unstained frozen and dried tangential section, ×20, *K*, keratin, *C*, epidermal cells, *D*, dermis

Fig 2. Human skin, unstained frozen and dried vertical section, ×20; *K*, keratin, *Ep*, epidermal cells, *D*, dermis.

enzymic activities of the epidermal cells exceed those within the dermis. That keratin possesses significant enzymic activity was somewhat surprising.

The activities of malic and lactic dehydrogenase in human skin by our methods[6] are 1000 to 3000 times larger than the results obtained with earlier methods employed in the analysis of skin of the rat.[7]

Table 1. Enzyme Activities of Three Layers of the Sole

	mM. substrate/kg. dry weight/hour (means)		
	Keratin	Epidermal cells	Dermis
Energy-producing: pathways of carbohydrate oxidation			
6-Phospho-gluconic dehydrogenase (aerobic—hexose→pentose→triose)	<20	510	170
Aldolase (anaerobic—hexose→triose→pyruvate)	<20	390	110
Lactic dehydrogenase (lactic→pyruvic)	290	>1010	no analysis
Fumarase (aerobic) } pyruvate to $CO_2 + H_2O$	<20	1800	600
Malic dehydrogenase } pyruvate to $CO_2 + H_2O$	610	>2000	no analysis
Non-energy producing:			
Acid phosphatase	620	1060	220
Alkaline phosphatase	<11	50	190
Purine nucleoside phosphorylase	110	216	<10

Table 2. Enzymes of Burn and Normal Epidermis

	mM. substrate/kg. dry weight/hour		
	Case 1	Case 2	Case 3
Acid phosphatase			
Normal	1,850	1,700	2,200
Burn	1,870	1,390	2,050
Glucose-6-PO_4 dehydrogenase			
Normal	687	712	563
Burn	609	735	523
Aldolase			
Normal	318	300	350
Burn	130	286	400
Malic dehydrogenase			
Normal	10,520	9,380	
Burn	12,200	14,400	

The very high activities of fumarase, malic dehydrogenase and lactic dehydrogenase in the cellular layer may well signify the preponderance of aerobic metabolism in skin.

From dried and unstained sections of the burns prepared in the same fashion the keratin was dissected and the analyses run on the epidermis and subjacent dermis of the burn and control (Fig. 2). These data are presented in Table 2.

DISCUSSION

The analyses thus far show no significant difference between burned and normal skin. This is surprising and somewhat disappointing, but does not exclude interference with other vital enzyme systems, and if these data are extended and confirmed they will point out the way for future work.

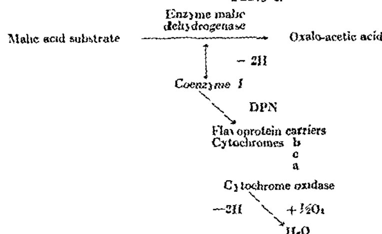
For example, the enzyme malic dehydrogenase is exceedingly rich in

Table 3. 6-Phospho-gluconic Acid Dehydrogenase in Skin

	MM./KG./HOUR		
	MEAN	SEM	CRITICAL RATIO
Epidermis	280	20	
			5.0
Sebaceous glands	750	70	
			4.2
Sweat glands	330	56	

Statistical tables show less than 1 per cent probability that these differences are due to chance

Table 4.



epidermis and may be one of the main energy-yielding pathways of oxidation. The hydrogen which is removed from the malic acid is added to oxygen after a long series of steps through nicotinamide coenzyme DPN and cytochromes. Peters[8] reports that exposure of homogenates to the same temperature for the same time we used for our burns caused loss of 71.4 per cent of the oxygen uptake with malic acid substrate. We have shown the presence of this enzyme in human epidermis and our data from burned skin suggest that the other product of the reaction, oxalo-acetic acid, is formed in normal amount. If confirmed, this indicates that the enzyme

activity of the system is intact up to this point and the decreased oxygen uptake Peters observed after heat may result from interference with these later steps in the electron transport chain, or with oxidative phosphorylation.

If future work shows a biochemical lesion in burns, possibly a simple enzyme analysis might predict which areas of burns would be third degree and thus be a guide for their early excision and graft. The activity of eight enzymes is preserved in frozen and dried skin. The clinical usefulness of non-viable frozen and dried skin "grafts" which has been reported[9, 10] might be partly due to active enzymes. More knowledge of the enzyme mechanisms may be useful for skin preservation and banks, and might make possible the culture of epidermis on a large scale.

In summary, quantitative micro methods have been applied to the study of eight enzymes of skin. The distribution of these enzymes in the separate layers of the sole is described. Seven of these enzymes (6-phospho-gluconic dehydrogenase, purine nucleoside phosphorylase, aldolase, fumarase, malic dehydrogenase, lactic dehydrogenase and acid phosphatase) previously unknown in human epidermis have been measured quantitatively. Three scalds were made with water at 50°C. for five minutes and removed one-half to one hour after injury. Analysis of scalded and normal epidermis showed no significant change in aldolase, glucose-6-phosphate dehydrogenase, malic dehydrogenase or acid phosphatase.

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LYMPHATIC LIPID ALTERATIONS IN THERMAL INJURY*

An Experimental Study

J. THOMAS PAYNE AND KATHRYN KRAUEL

The effect of thermal injury on the living animal and on the various tissue components of the animal has been studied for some time. Changes in tissue and circulating proteins, electrolytes, and cellular elements have been fairly well defined following burns. Changes in other major tissue components have been postulated. The possibility of significant changes in the lipid component was suggested by the thermolability of lipids. With this in mind a specific study of lipid alterations in thermal injury was initiated. The problem was approached from three ways: (1) chemical analysis of body fluids leaving the burned area, including lymph and blood, (2) analysis of the local lesion by microchemical, histochemical and isotopic techniques, and (3) biological testing of suspicious lipids isolated by these studies. The study of the lipid component changes in lymph draining from the burned area was selected as the first approach, for technical reasons. It was felt that lymph would reflect the earliest changes in fluids escaping from the burned area and at the same time be most readily amenable to screening lipid analyses.

METHOD

Large mongrel dogs were used. The animals were anesthetized with intravenous Nembutal One hind leg was clipped, and with clean technique a parasaphenous lymphatic was cannulated with a No. 20 polyethylene catheter. The saphenous vein and adjacent lymphatics were ligated. Lymph was collected in graduated cylinders containing 1000 U of crystalline heparin. Collection periods were for 20 minutes, the foot of the animal being squeezed every 15 seconds. Ten such samples were collected. The animals were permitted to recover One week later a similar lymphatic in the other hind leg was cannulated. Several normal lymph samples were collected, then the foot was immersed up to the hock in water of 70°C for one minute Ten consecutive 20 minute samples of lymph were collected just as from the control extremity.

The lymph was lyophilized, then analyzed in sequence for total lipid, lipid phosphorus, cholesterol and glycerol. The preliminary extraction of lipid was made by cold chloroform-methanol 2·1. Total lipid was determined by weight of extracted material. Lipid phosphorus was determined by a dry ash modification of the procedure described by Hoagland[1] The cholesterol method of Sperry[2] was used. Glycerol was determined by the method of Voris et al.[3]

These analyses have been done on a total of 10 dogs, with variations in

* From the Department of Surgery of the School of Medicine, University of Washington, Seattle, and the Seattle Veterans Administration Hospital This investigation was supported by the Medical Research and Development Board, Office of the Surgeon General, Department of the Army, under Contract DA-49-007-MD-419.

the lipid components measured depending on the volume of lymph obtained per sample. Seven of these were subjected to burns.

RESULTS

The volume of lymph obtained in the control samples varied from 1 to 3 cc. and was related to the size of the dog, the lymphatic size, and to the degree of squeezing of the foot. After the burn, the flow of lymph was copious and ranged as high as 17.5 cc. per 20 minute period, but averaged about 8.0 cc. The increment was in the first 20 minute period. This paralleled the observations of Cope and his co-workers,[4] and others.

The total lipid per milliliter showed no significant variability due either to the trauma of prolonged cannulation or to the thermal injury. The average total lipid varied between 2.25 and 3.75 mg. per milliliter of lymph. With the precipitous increase in volume of lymph flow, however, there was an increase in total lipid moved by the lymph (Fig. 1).

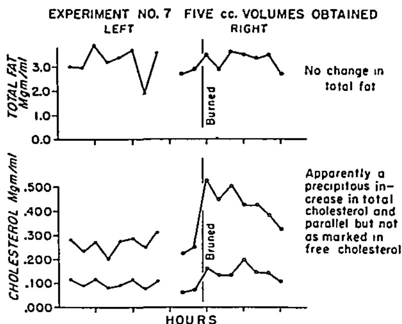


Fig. 1. The total fat in lymph does not seem to change markedly after burning; however, the cholesterol, both total and free, in this experiment rose precipitously. The drop-off after two hours was not a uniform finding.

The cholesterol showed parallel changes in both free and total components. Again, no significant alteration due to trauma was noted. The reaction to burning of the foot was an immediate rise in the total cholesterol and a less striking rise in the free cholesterol. The order of change for the total cholesterol was from the normal of 0.25 mg. per milliliter to 0.5 mg. per milliliter, and for the free cholesterol was from 0.10 mg. per milliliter to over 0.15 mg. per milliliter (Fig. 1).

The neutral fat, determined as glycerol, showed a similar suggestive increase after burning; however, the volume of the lymph samples obtained has not permitted a sufficiently large number of determinations to be certain the trend is not a matter of chance (Fig. 2).

The concentration of lipid phosphorus per milliliter in the control samples likewise showed no change due to prolonged lymphatic cannulation and foot squeezing. However 20 minutes after the burn the concentration per milliliter rose from an average of about 0.014 mg. in the control samples to over 0.03 mg There was a sustained elevation without any definite decrease within three hours after the burn (Fig. 2).

DISCUSSION

There are many factors to control in lymph collection, burning, and subsequent analyses. The role of the anesthesia on lymph formation, the trauma of squeezing the foot, the uncertainties of the daily lipid intake of

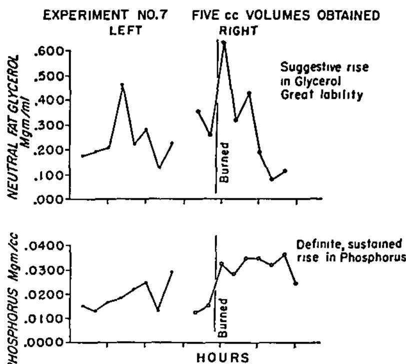


Fig 2 The change in the neutral fat, as glycerol found in the lymph after burning, was too labile for certainty in the small series The lipid phosphorus levels, however, consistently showed this much or an even greater increase.

the animals—all may serve to produce a false lipid picture. Nevertheless, the consistent findings typified by the graphs serve to confirm the reasonable expectation that lipid changes do occur after thermal injury.

Others have found suggestive evidence of lipid alteration after burns. Fazekas and Bacsich noted an increase in lipoid bodies in the leukocytes five hours after a burn These bodies disappeared in 5 to 6 days unless the patient became moribund, in which case they increased precipitously.[5] Recently Thomas, Vaughan, Walker and Pace found an increase in circulating low density lipoprotein in dogs after burns of 20 per cent.[6] The altered lipid proportion of lymph appears to be a logical finding in view of these observations.

It would be premature to speculate too much on the significance of lipid changes in burns. It is known that certain free lipids—particularly phospholipids—are capable of producing a hemolytic anemia when injected par-

enterally into animals. Thompkins[7] and her co-workers demonstrated this and the tissue reaction to lipids, which is similar to the tissue reaction in a burn. The specificity of the lipid reaction may be open to question; nevertheless, it falls within the cyto-irritative group of reactions.

The lipid changes in lymph collected from the burned extremities are of such a magnitude as to warrant further study. Lipids, thermally degraded in the burned area, may be released into the circulation and be traceable as to origin and fate. They well may play a role in producing a specific local cellular response as well as a systemic hematologic reaction.

SUMMARY AND CONCLUSION

Analysis of lymph draining from thermally injured dog legs shows significant lipid changes. There is an increase in volume of lymph, but the concentration of total lipid per milliliter remains fairly constant. There is a definite abrupt rise in cholesterol, lipid phosphorus and, to a degree, glycerol after burning. These findings suggest a further examination of the local burn for lipid changes and a study of the reaction to the specific lipids involved.

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THE ROLE OF EPINEPHRINE IN HYPERKALEMIA OF ACUTE EXPERIMENTAL BURNS*

CAPTAIN CLARKE L. HENRY (M.C.)**

It has been clearly established that hyperkalemia occurs in the early phase of traumatic, hemorrhagic, and burn shock.[1-4, 10] The purpose of this study was to investigate the mechanism involved in the production of hyperkalemia during the acute phases of thermal injury. Although local tissue destructive processes with potassium release, as encountered in trauma and

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** With the technical assistance of Raymond J. Lichter, B.S., and John C. Daw, B.S. The author wishes to express gratitude to Melvin S. Schwartz, M.D., who gave generously of time and effort in the presentation of the statistical data of this paper.

burns, may play the major role in the production of hyperkalemia, this does not preclude an over-all humoral control mechanism mediated via the "stress response."

Specifically, the well documented interrelationships between epinephrine and potassium suggest that perhaps the focus of investigation in re the hyperkalemic mechanism should center not primarily on adrenocortical function, but instead should concern itself more directly with the medullary component. There are many sound observations which lend rather convincing support to this concept.

Stimulation of the sympathetic nervous system has been shown to produce increasing concentrations of serum potassium.[5] D'Silva and others have demonstrated abrupt, transient increases in serum potassium following intravenous injections of epinephrine.[6 9] In pheochromocytoma, with a persistent type of hypertension due to circulating epinephrine, associated serum potassium levels of 10 to 12 mEq. per liter are a matter of record. Hemorrhagic shock, with increased circulating epinephrine, is accompanied by an increase in plasma potassium concentration. In this instance, hyperkalemia does not occur if the adrenal glands have been previously tied off; one may infer that the removal of the epinephrine source is the key factor. Intravenous epinephrine in an adrenalectomized animal will also produce hyperkalemia; this is evidence that the effect of epinephrine is not one directly upon an intact adrenal gland.

There can be little doubt that increased circulating epinephrine will mobilize potassium and produce hyperkalemia. This premise is the basis for the following hypothesis: In trauma an increase in circulating epinephrine, as part of the over-all stress response, produces the associated increases of plasma potassium concentrations which have been observed. One might then reasonably expect that the administration of an adrenolytic substance to a known hyperkalemic burn preparation should, if the mechanism is one of epinephrine release, result in a suppression of the hyperkalemic pattern

METHODS

A definite reproducible response pattern of hyperkalemia to a standardized burn has been established; the technique, subsequent blood sampling procedures, and resultant pattern have been previously reported.[10] Briefly, this is an immersion burn of the left hind leg in water at 100°C. for 30 seconds. The standard burn series in this present report consists of a duplicate group of 10 mongrel dogs, subjected to the standard immersion burn and post-burn blood sampling procedure via parallel femoral artery and vein of the burned limb An additional 10 animals were also subjected to this standard burn procedure, however, immediately following the 10 minute post-burn arterial and venous samples, piperoxan hydrochloride,* in the amount of ½ mg. per kilogram body weight, was administered intravenously. All plasma potassium analyses were by flame photometry.† All data, recorded as mEq per liter, were subjected to statistical evaluation by analysis of variance,[11] and mean values of plasma concentrations were plotted on arithmetic graph paper versus time

* Benodaine hydrochloride, registered trademark for Merck's brand of piperoxan hydrochloride.

† Perkin-Elmer Flame Photometer Model 52-C.

Figure 1 represents a control series for the standard burn.[10] It consists of a mean of 5 animals, not burned, yet subjected to the anesthesia and serial blood sampling procedure. It will be noted that the respective curves for venous and arterial potassium concentration are essentially flat and superimposed. Statistically, there is no difference between the two blood

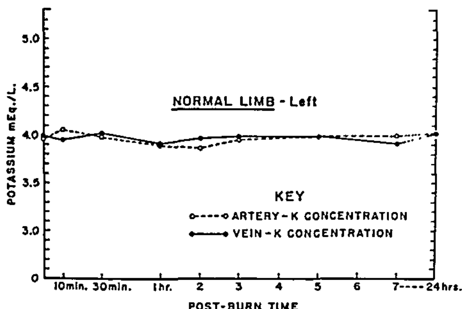


Fig. 1. Mean potassium concentrations, normal control series.

vessels sampled with respect to artery or vein or to time interval at which blood was withdrawn ($P > .05$).

Figure 2 presents a graphic summary of the 10 animals of the standard burn series. There are gradual but definite over-all rises in plasma potassium concentrations in both arterial and venous channels. A concentration

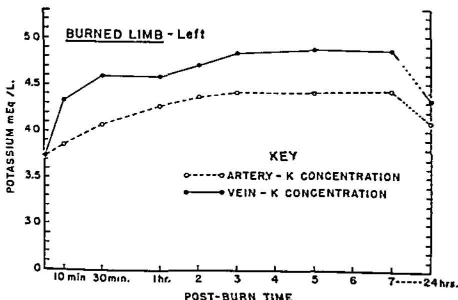


Fig. 2. Mean potassium concentrations, standard burn series.

gradient of significance (P < .001) develops between the parallel artery and vein.

Comparison of this response pattern with the piperoxan burn series (Fig. 3) reveals an initial similarity until the 10 minute post-burn interval just prior to the drug. The concentration gradient between artery and vein at this point is statistically significant (P < .001). Following piperoxan, there is a sustained suppression of the hyperkalemic pattern, and a concomitant amelioration of the concentration gradient between artery and vein.

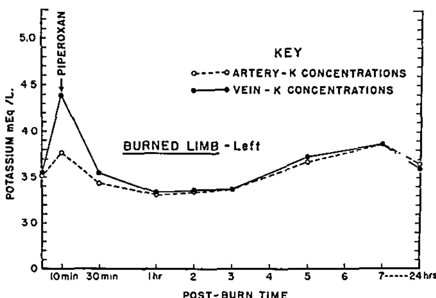


Fig 3 Mean potassium concentrations, piperoxan burn series.

DISCUSSION

The pattern of plasma potassium concentrations following piperoxan administration (Fig 3) is in sharp contrast to that obtained in the standard ... an ... ous ... by piperoxan lend considerable support to the hypothesis advanced earlier, namely, that hyperkalemia of acute thermal injury is mediated via humoral mechanisms involving increased circulating epinephrine. The initial venous potassium peak during the first 10 minutes post-burn, and its suppression thereafter by the adrenolytic drug, suggest that the site of action is at least in part a local one at the cellular level.

Potassium hypersensitivity is seen in the shocked animal This is a proven status of selective potassium toxicity and in magnitude may exceed 6 to 9 times that of normal[12] The remarkable protective action of Dibenamine on animals subjected to traumatic and hemorrhagic shock procedures is well documented, though less well understood[13 15] Perhaps this protection is manifest by the adrenergic blocking property of the drug, indirectly preventing a deleterious hyperkalemia via suppression of circulating epinephrine.

SUMMARY

1. Piperoxan hydrochloride effectively suppresses hyperkalemia developing during the first 24 hours in acute experimental burn shock.

2. It would seem that increased circulating epinephrine as one facet of the stress response to thermal trauma is responsible, at least in part, for hyperkalemia observed in the acute experimental burn.

3. It is further suggested that the protective effect of Dibenamine in the shocked animal may be one of adrenergic blockade, preventing an otherwise lethal hyperkalemia in a shocked preparation known to be thereby hypersensitive to even moderate increases in potassium blood levels.

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THE AMINO-ACIDURIA OF TRAUMA*

GEORGE L. NARDI

The negative nitrogen balance of the post-traumatic state, though well established, remains a collective term whose meaning is poorly understood.

In order to better define this loss, a chemical partition of the urinary nitrogen of traumatized patients was undertaken.

Paper chromatography was utilized to study the behavior of the urinary amino acids, one of the subdivisions of urinary nitrogen These studies suggested that an amino-aciduria, consisting of the excretion of essential

* From the Department of Surgery of the Harvard Medical School at the Massachusetts General Hospital, Boston These studies were aided by financial support from Departments of the Army and Navy, NR 114-198.

amino acids not normally present in urine as well as increased quantities of non-essential amino acids, contributed to the nitrogen loss of trauma.[1]

METHODS

In order to quantitate these observations more precisely the gasometric method of Peters and Van Slyke[2] was utilized in a series of patients suffering thermal trauma. The result of this study, which confirms the preliminary chromatographic observations, is the subject of this report.

The maximum normal excretion of free amino acids in man is approximately 200 mg. per 24 hours. To confirm this value with our techniques, urinary collections were made on 6 members of our laboratory staff on 5 consecutive days. No change was made in their dietary habits or daily activities. Figure 1 demonstrates the 24 hour excretion of these "normal" individuals to be well below the accepted maximum of 200 mg.

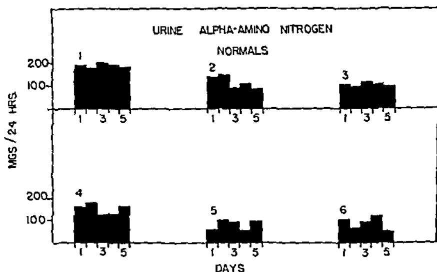


Fig. 1.

Five severely burned patients were studied. All were brought to the hospital shortly after injury. All had suffered extensive thermal trauma involving from 16 to 60 per cent of the total body surface. Over one-half of the total burn was full thickness in each instance. Treatment was similar in all cases Plasma and blood were used for colloid replacement according to a standard formula[3] Penicillin and streptomycin were administered in every case. All the burned areas were treated with occlusive dressings. Administration and calculation of oral intake were supervised by a special dietitian. Twenty-four hour urine samples were collected in bottles containing thymol and toluol by means of an indwelling catheter and kept under refrigeration. Aliquots were used for the analyses.

The results are summarized in Figure 2. In this chart the 24 hour amino acid excretions are plotted horizontally for each individual patient. The latter are arranged from above downwards in order of increasing severity of the burns. The figure at the right of each horizontal line represents the percentage of total body surface burned. As can be seen, there is a conspicuous hyperexcretion of amino acids in the urines of these patients. This increased

excretion is at a peak the first week or two after the burn and then slowly subsides, but may not return to a normal range until convalescence is completed and the burned areas completely resurfaced.

DISCUSSION

These findings parallel our previous observations with paper chromatography.[1] The latter method had the advantage of demonstrating a qualita-

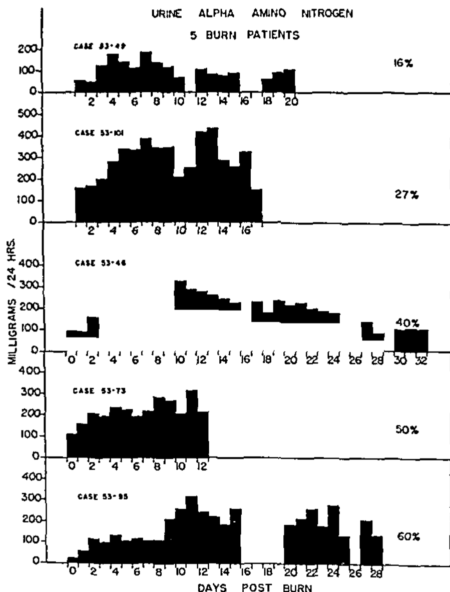


Fig 2

tively different pattern in the amino-aciduria of trauma. The gasometric method utilized in these studies affords no qualitative testimony but does prove the quantitative existence of amino-aciduria subsequent to thermal trauma.

The mechanism of this amino-aciduria remains unclear. Gross kidney function as measured by volume output, concentrating ability, urinalysis,

amino acids not normally present in urine as well as increased quantities of non-essential amino acids, contributed to the nitrogen loss of trauma.[1]

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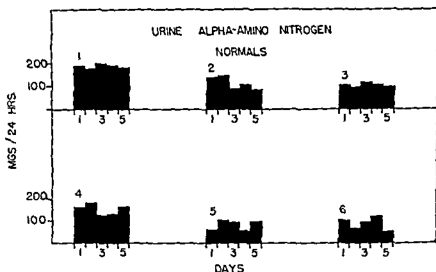


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centage of total body [illegible]

hyperexcretion of amino acids in the urines of these patients. This increased

the post-traumatic phase may present the renal tubule with a greater reabsorptive load than it can handle.

The urine of the burned patients was accordingly subjected to a complete chemical partition of its nitrogen constituents (Fig. 4). It was found that all were greatly increased, particularly the urea and "undetermined nitrogen" fractions.

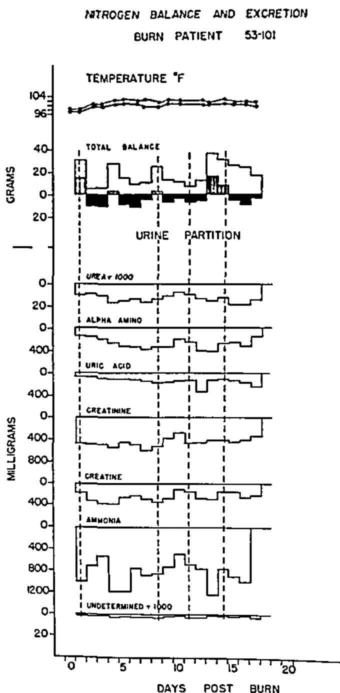


Fig. 4.

and blood non-protein nitrogen was normal. Since the amino acids are freely filtered through the glomerulus and depend on tubular reabsorption for their retention, it seems most likely that some form of failure of the normal process of tubular reabsorption occurs to account for this escape of amino acids.

Such a failure of tubular function could result from primary tubular injury per se, from overloading as a result of hyperamino-acidemia, or from overloading by other nitrogenous fractions presented to the kidney for excretion.

Since gross kidney damage was not detected it seems unlikely that significant, primary tubular damage had occurred.

Repeated determinations of plasma amino acid levels failed to disclose

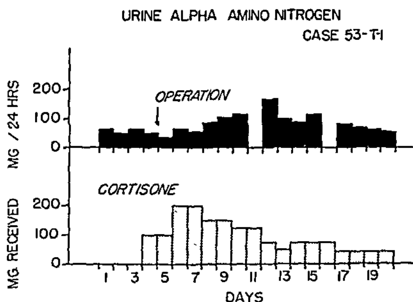


Fig 3

significant elevations so it is unlikely that hyperamino-acidemia is a significant factor

The possible role of adrenal steroids in influencing tubular function must be considered in traumatic states.[4-7] In order to assess the role of adrenal secretions, patients undergoing total or subtotal adrenalectomy were studied. Previous observations[8] indicated that the magnitude of surgical trauma inherent in surgical adrenalectomy should result in obvious amino-aciduria

Two patients with metastatic malignancy of the breast who underwent transabdominal total adrenalectomy and two patients with Cushing's disease who had radical subtotal adrenalectomy failed to show the expected degree of amino acid excretion (Fig 3) These patients were maintained on large doses of cortisone during their immediate postoperative period.

These observations suggest that the presence of intact adrenals is necessary for post-traumatic amino-aciduria. It is possible, on the other hand, that the large doses of cortisone may have increased tubular reabsorption.[9, 10]

The presence of an excess of all the nitrogenous constituents of urine in

istration of whole blood and salt water are in controversy. A possible resolution of this problem may come from knowledge which correlates volume disturbances in severe burns with other physiologic variables such as internal rates of exchange, pulmonary and renal function and, finally, survival. In this report the interrelated volume changes and water exchange rates which attend severe burns are described.

METHODS

Studies were conducted on human beings and dogs. Normal human subjects and patients suffering from lethal burns involving 30 to 80 per cent of body surface of deep second and third degree were employed. The methods employed in human subjects have been outlined in a previous report.[1] Healthy mongrel dogs were subjected to light pentobarbital anesthesia and the femoral veins and arteries exposed under aseptic conditions. Standard amounts of deuterium oxide, T-1824, radioactive S^{35} and radiochromium^{51} tagged red cells were injected intravenously and arterial samples withdrawn at graded periods extending to three hours. Following this, the animal was returned to his quarters and allowed to recover. After an interval of three to seven days, the animal was again anesthetized and subjected to a 70 per cent deep burn by (direct flame early in the study) immersion in scalding water (85°C.) for one minute. Approximately one hour post-burn, the studies utilizing T-1824, radioactive S^{35}, radiochromium^{51}, and deuterium oxide were repeated. The animal was then returned to his cage and observed. No therapy was employed. The extent of burn has been determined by tracing the burned portion with cellophane, determining the area and then applying the Cowgill-Drabkin formula for body area in the dog.

Deuterium oxide content was determined in the water of lyophilized whole blood employing the falling drop method. Radioactive S^{35} content was determined utilizing a Q-gas flow chamber and Geiger-Müller counter. Radiochromium^{51} was determined with the use of a scintillation counter with a liquid gamma attachment and a standard scaling unit.

RESULTS

Human Studies. Studies on 9 normal subjects and 10 burned patients are presented in this report. Of the 10 burned subjects, only one survived. The data relative to survival and the time the studies were performed are presented in Table 1.

The results of the studies on internal water exchange rates and compartmental volumes in normal and burned subjects are presented in Figure 1. Extracellular fluid volume as measured with radioactive S^{35} ranged from 9 39 per cent to 22.6 per cent (average 16.9 per cent) body weight in normal subjects. In burned patients extracellular S^{35} volume ranged from 8.1 per cent body weight to 22.8 per cent body weight (average 14.2 per cent). The difference between the means is probably significant, for P is less than .02. This indicates that during the early stages of a severe burn there is a 15 per cent mean reduction in extracellular fluid volume as measured with radioactive S^{35}. The clinical fact that large amounts of extracellular fluid are lost into and about an area of burned tissue is well known. Measurements in burned dogs of extracellular fluid volume obtained with radiosulfate tend to substantiate this clinical finding. These findings suggest that the measurement of volume of distribution (V_D) of radioactive sulfate may

CONCLUSIONS

Amino-aciduria is a part of the negative nitrogen balance of severe trauma. This escape of amino acids is not the result of renal damage and probably is secondary to overloading of the renal tubules by nitrogenous compounds. However, the adrenals may also play a role since adrenalectomized patients maintained on cortisone do not demonstrate this amino-aciduria.

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BLOOD, EXTRACELLULAR FLUID AND TOTAL BODY WATER VOLUME RELATIONSHIPS IN THE EARLY STAGES OF SEVERE BURNS*

MORRIS J. FOGELMAN AND BEN J. WILSON

The present status of fluid therapy during the early phases of a severe burn has been engendered by clinical and experimental studies of compartmental changes in blood, plasma, extracellular fluid and total body water volumes. Data gathered from burned patients and experimental animals indicate that a severe burn results in an early reduction in salt water volume (extracellular fluid) and a variable loss of red cell mass within the body. Although the qualitative losses of extracellular fluid and red cell mass are generally accepted as characteristic changes which attend a severe burn, therapeutic corollaries which quantitate the relative order of needs for the early admin-

* From the Department of Surgery, Southwestern Medical School of The University of Texas, Dallas This investigation was supported by the Medical Research and Development Board, Office of the Surgeon General, Department of the Army, under contract number DA-49-007-MD-402.

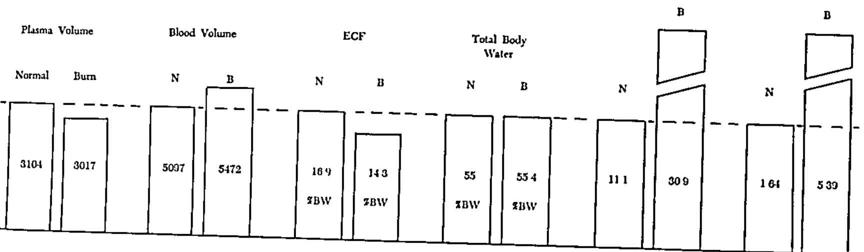


Fig 1 Mean compartmental and exchange rate values compared in groups of normal and burned men The mean body weight in the ten burned patients was 70 8 kg, whereas the mean weight in the twelve normal individuals was 72 8 kg %BW = Per cent Body Weight.

be an important method whereby one may not only quantitate the amount of extracellular fluid which has been lost externally in certain abnormal states, but one may also use this method as an indication of the amount of distributional shifts of salt water which occur *within* the body. Subsequent investigations will have to be performed to determine whether this property of S^{35} V_D actually exists in other states of induced ECF distributional shifts.

The results of the measurement of blood volume (T-1824–hematocrit method) in normal and burned subjects are also presented in Figure 1. There is a difference in the mean blood volume and plasma volume during

Table 1. Data on Burn Patients

PATIENT	AGE	BURN % BODY SURFACE	SURVIVAL	AMOUNT OF BLOOD RECEIVED PRIOR TO STUDIES	TIME STUDIES PERFORMED (HOURS POST-BURN)
9 J F	20	70	Died on 10th day	1000 ml.	6th day
10 R.L	33	60	Died on 6th day	500 ml.	19 hours
12 M M	41	65	Died on 10th day	0	3 hours
18 H J	40	40	Alive and well	0	29 hours
20 T.H.	30	80	Died, 55 hours	0	8 hours
21 B H.	25	80	Died, 56 5 hours	0	11 hours
23 J R.	90	30	Died, 36 hours	0	16 hours
24 M C	41	50	Died, 11 hours	0	5 hours
25 P R	65	35	Died, 57 hours	0	19 hours
26 A L	21	40	Died, 10th day	500 ml.	24 hours

the early stages of severe burns when compared to normal, but these differences are not statistically significant ($P > 05$). Seven of the 10 burned subjects received no blood prior to the volume determinations. It is recognized that hemolysis may interfere with the determination of blood volume when the dye method is used, and that the error is not of predictable direction—that is, the colorimetric reading may be minimized or intensified, depending upon the relative concentrations of plasma dye and plasma hemoglobin. Further, it has been shown by Cope and Moore[2] that there is free escape of plasma protein from the vascular tree following thermal injury. Since the protein binds the blue dye, a given diminution in circulating plasma volume would be falsely minimized as a result of the increased V_D of plasma protein.

Table 2. Water Exchange Rates in Burned and Normal Dogs

DOG NO	WGT IN KG	EXTRACELLULAR S^{35} VOL. PRE-BURN %BW	EXTRACELLULAR S^{35} VOL. POST-BURN %BW	TRANSCAPILLARY WATER EXCHANGE* PRE-BURN	TRANSCAPILLARY WATER EXCHANGE* POST-BURN	TOTAL BODY WATER PRE-BURN	TOTAL BODY WATER POST-BURN	% BODY SURFACE BURNED	SURVIVAL IN HOURS
1	14.09				9.14	46.4	44.4	70.0	†
2	13.6	18.8	8.9	4.0	3.0	63.9	46.9	33.1	120
3	15.4			2.5		46.4			0
4	12.0	36.6	14.83	2.3	3.7	53.4	67.0	38.4	5.0
5	11.8	25.2	18.6	2.9	2.9	57.0	48.7	33.8	>120
6	15.4	25.9	12.5	2.9	4.6	46.5	47.5	61.0	12.0
7	11.05	44.8	31.2					26.1	14.5
Average:		29.2	17.2	2.9	4.66	52.3	50.9		

*Percentage of body water which passes across the functional capillary area per minute.
†A 70 per cent body surface direct flame burn. Animal sacrificed upon completion of studies.

The results of the studies on other compartmental water volumes and internal water exchange rates are presented in Figure 1. Mean total body water volumes as determined with D_2O are essentially the same in burned patients (55 4 per cent body weight) and in normal subjects (55 0 per cent body weight). This finding is not unexpected, and it indicates that no significant external losses have occurred and that the deficit of ECF as measured with S^{35} has come about as the result of a shift of water into the cells; or the alternate hypothesis is that radioactive sulfate does not diffuse into the edema fluid of the burned part, even though it is extracellular, while D_2O does. That D_2O diffuses freely into a burned part is likely, for after burning the transcapillary diffusion rates of D_2O are increased about 200 per cent. In order to know how to interpret these findings with certainty it will be necessary to study differential diffusion rates of D_2O and sulfate into edematous pools having various ionic concentrations.

If it may be conjectured that the increment in transcapillary exchange of water, from 1 64 per cent of the body water across the capillary area in either direction per minute in normals to 5 39 per cent after burns, occurs primarily in burned parts, then it follows that the non-burned areas may suffer a relative reduction in the rate of capillary exchange. Hence, the whole body (liver, lungs, heart, adrenals, etc) may suffer as the result of a redistribution of capillary area available for exchange in favor of the burned area That there is a definite change in capillary function in an area of burned tissue is supported by the works of others. Drinker et al.[3] have found an increase in lymph flow and protein concentrates from the burned leg of an animal. Cope and Moore[2] have studied capillary permeability in burns, and found that an increased concentration of radioactive colloid appeared in the lymph from the burned extremity of a dog.

Dog Studies. The results of studies on internal water exchange rates and compartmental volumes in normal and burned dogs are presented in Table 2 There appears to be no consistent change in total body water volume in dogs following burning Admittedly the series is too small to arrive at any definite conclusions, but the results generally parallel those which obtained in the human subject Extracellular S^{35} fluid volume is greatly reduced following a severe burn. In the normal state, the S^{35} volume is an average of 29 2 per cent body weight, while after burning it falls to 17 2 per cent body weight, a reduction of about 40 per cent of effective extracellular fluid. There are insufficient data to correlate the change in extracellular fluid volume with the severity of burn or survival.

The results for internal rates of water exchange in normal and burned dogs are also presented in Table 2. The results indicate that in the normal dog from 2.3 to 4 0 per cent (average 2 9 per cent) of blood water moves across the capillary area per minute. These rates in the dogs are much slower than those previously reported by Fogelman[4] The reason for the variance is that the calculated rates from the present study have been obtained from the middle portion of the disappearance curve for D_2O in blood water (6 to 10 minutes) as it approaches an asymptote, rather than from the first portion of the curve (2 to 8 minutes) as previously reported in the dog. The reason for the change is that less variation will occur from consecutive determinations on the same animal and from one animal to another, since the later segment of the curve more nearly represents an equilibrium state. Following a severe burn, from 2 9 to 9 14 per cent (aver-

age 4.66 per cent) of blood water moves across the capillary area per minute. In other words, following burning there is a 60 per cent increase in the transcapillary rate of movement of water. The results tend to parallel those found in human burns; that is, an increase in total capillary area available for exchange attends a severe burn. The number of animal experiments reported is too small to derive any correlations between rates of exchange and survival.

Comparative data on blood volume studies utilizing T-1824 and radioactive chromium[51] are presented in Table 3. The T-1824 plasma and blood volumes fell in 3 of 4 experiments in which before and after burning determinations were made. The mean blood volume decreased 11 per cent solely as a result of the fall in plasma volume. The radiochromium plasma and blood volumes fell in every instance after burning. The mean blood volume decreased 23 per cent as the result of the fall in plasma volume. In this small series the T-1824 volume decrement after burning tends to be smaller than the radiochromium volume decrement, and in dog No. 6, the most severely burned, the T-1824 blood volume increases. This paradoxical result was observed in the clinical study.

SUMMARY AND CONCLUSIONS

1. During the early phase of burns in man there is a mean reduction in extracellular S^{35} fluid volume (approximately 15 per cent) as compared to a group of normal subjects. The reduction in extracellular fluid volume while total body water volume (D_2O volume of distribution) remains relatively unchanged after a burn is evidence of a compartmental translocation of salt water from an extra- to intracellular residence, or of restriction of S^{35} movement from the fluid that has been sequestered in the burned parts.

2. There appears to be little significant change in mean plasma volume and blood volume in burned patients compared to normal subjects when determined by the T-1824–hematocrit method. Studies with radioactive chromate indicate that the blood volume is regularly reduced in the experimental animal after burning.

3. The transcapillary rates of movement of water in human beings and dogs are significantly accelerated after thermal trauma (over 200 per cent of the normal rates in man). It is thought that the rate increment represents an increase in functional diffusional area in the body, but whether the increment is due to altered exchanges in the burned part or in the uninjured parts of the body, or both, is not critically shown in the reported experiments.

4. Further study is necessary to define the characteristic changes in blood volume, extracellular fluid, total body water and internal rates of water exchange with graded burns in dogs and chance burns in man; and to correlate these alterations with survival and the effect of therapy.

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Table 3. Comparison of Blood Volume Changes in Dogs

DOG NO	HEMATOCRIT		T-1824 METHOD: PLASMA VOLUME (ML.)		T-1824 METHOD: BLOOD VOLUME (ML.)		RADIOACTIVE CHROMATE METHOD: PLASMA VOLUME (ML.)		RADIOACTIVE CHROMATE METHOD: BLOOD VOLUME* (ML.)	
	PRE-BURN	POST-BURN	PRE-BURN	POST-BURN	PRE-BURN	POST-BURN	PRE-BURN	POST-BURN	PRE-BURN	POST-BURN
2	45.5	50.0		476		952	695	470	1275	1065†
3	37.0		500		793		1026		1630	
4	40.0	48.5	750	600	1250	1165	762	358	1271	734
5	42.0	44.5	800	464	1379	830	776	505	1339	948
6	41.0	50.0	615	616	1042	1232	838	595	1421	1233
7	35.0	49.0	750	400	1153	784	986	700	1518	1415
Average.	40.0	48.4	683	511	1123	993	847	525	1408	1078
Mean change after burning						−11%				−23%

*The volume of blood removed for sampling between "pre- and post-burn" has been added to the post-burn blood volume so the effects of blood removal will not be confused with the effects of burning. This added volume is not used in computing plasma volumes.

†Twenty-four hours after this determination only 82 per cent of red cell mass survived

The changes in sodium space were determined by division of the period (2 to 7 days) sodium balance by the serum sodium concentration.

The change in the chemical spaces was estimated on the first determination as the difference between the observed value and the normal value for these spaces predicted from the control group. Actual difference values were used after the first determination.

The patients were weighed in the morning. Corrections were made for

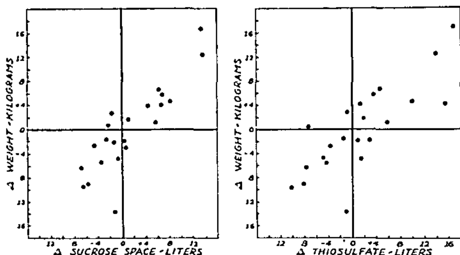


Fig. 1 Δ weight versus Δ chemical spaces.

the dressings present. When possible, fresh dressings were applied before weighing prior to the space determinations.

RESULTS

In Figure 1 is plotted the alteration in weight against the changes in the sucrose and thiosulfate spaces. A good correlation exists. Slightly less scatter is present in the Δ sucrose space than in the Δ thiosulfate space.

Figure 2 is a scatter plot of the changes in sodium space against the alteration in the chemical spaces. When the sodium space change is positive,

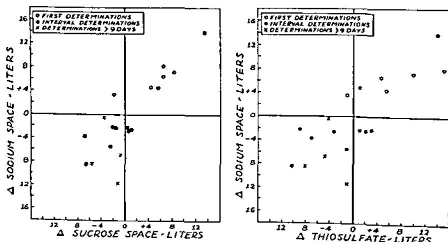


Fig. 2. Δ sodium space versus Δ chemical spaces.

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COMPARISON OF THE VOLUMES OF DISTRIBUTION OF SUCROSE AND SODIUM THIOSULFATE AS AN ESTIMATE OF EXTRACELLULAR FLUID IN BURNED HUMANS*

JERRY A. STIRMAN, JOHN F. PRUDDEN, AND M. KENDALL YOUNG, JR.

Clinical and animal experimentation have indicated the occurrence of compartmental water volume changes, and ionic transfers following severe burning.[1, 2] Recent work in this laboratory has indicated that previously applied methods of calculation of the volume of distribution of rapidly equilibrating substances used to estimate the extracellular fluid volume, as well as the identity of the measured space and the actual space, may be in error.[5, 6] This report deals with the results of use of the volumes of distribution of sucrose and sodium thiosulfate as estimates of the extracellular fluid volume and its changes following thermal injury The justification of the use of these chemicals depends upon the demonstration of reproducibility, accuracy, and practicality The reproducibility and practicality of the methods employing these substances have been established,[7] but the accuracy has not Gross changes in the extracellular fluid volume are indicated by (1) the clinical signs, (2) changes in weight, and (3) the change in sodium balance. The correlations which obtain between these variables and the alteration in the volumes of distribution of sucrose and sodium thiosulfate would indicate whether positive and negative loads are appropriately registered.

MATERIALS AND METHODS

Experimental. The twelve patients studied were young adults who were burned on 30 to 80 per cent of the body surface area. In all but one, a minimum of 30 per cent of the body surface area was involved with third degree burn. The first determination was performed from 0 69 to 2 5 days. One to three subsequent examinations up to 20 days after the burn were done on 8 patients for a total of 27 determinations. Complete metabolic balance data was available on 6 patients, representing 19 interval measurements.[4]

Analytical. The chemical methods used have been described previously.[7]

Calculation. In another communication,[5] the inadequacies of the method of extrapolation for the calculation of the volume of distribution of rapidly equilibrating substances have been pointed out. The total load method of calculation of the volume of distribution of sucrose and thiosulfate used in this paper is independent of these difficulties.

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following the injury, the weight change reasonably reflects change in the extracellular fluid volume. This conclusion would seem warranted, as the greatest change in weight is produced by the administration of fluids which remain predominately extracellular, and which are subsequently lost from the body.

2. If the weight change accurately predicts change in the ECF volume, then the alteration in the chemical spaces appropriately reflects this change.

The Δ sodium space versus Δ chemical space correlations indicates that:

1. When the sodium space increases there is an increase in the chemical spaces. The sucrose space increments reflect this in an approximate order of magnitude, the slope being slightly less than one. The thiosulfate space increment becomes inordinately large as the sodium space increases.

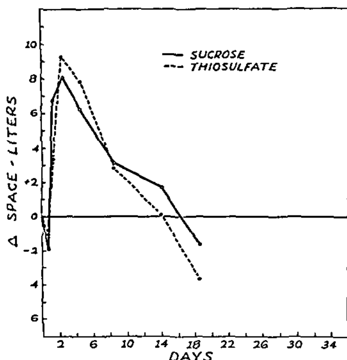


Fig. 4. Δ chemical spaces versus time after burning.

2. When the sodium space decreases, there is a poor relationship existing between this change and the change in the chemical spaces. The change is reflected only in direction, not in magnitude.

3. Considering the chemical spaces the more accurate, the change in sodium space as calculated does not reflect change in the extracellular fluid volume. Indeed, as much as 400 to 500 mEq. of sodium might be lost or gained without appreciably affecting the extracellular fluid volume. The demonstration of exchangeable sodium in large quantities in bone which could act as a reservoir might explain this discrepancy.[3]

The good relationship existing between the change in the sucrose space and change in thiosulfate space indicates their movement is parallel in direction, but not in magnitude. Since the thiosulfate space increases and decreases proportionately greater than sucrose, and does not correlate well with the Δ sodium space as calculated, a possible conclusion is that the thiosulfate ion may move intracellularly with sodium; consequently, its

there is almost unit for unit change in the Δ sucrose space, but disproportionate change in the Δ thiosulfate space. If the sodium balance is negative, there is very little correlation evident. If the crosses* representing the long period sodium balance figures are deleted from both graphs, there is a slight trend present. Additional determinations might clarify this trend.

The comparisons of the changes in the sucrose space against the changes in the thiosulfate space are presented in Figure 3. The slope of this fit is different from one, indicating that for any unit change in sucrose, there is a proportionately greater change in the thiosulfate space.

In Figure 4 are demonstrated the changes in the chemical spaces against time. There is a rapid increase in the spaces, reaching a peak between the

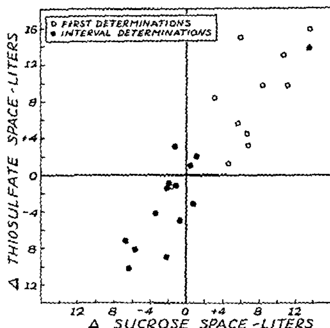


Fig 3 Δ thiosulfate space versus Δ sucrose space

second and third day, a gradual decline back to normal takes place between the twelfth to fourteenth days Thereafter, the Δ spaces become subnormal

The clinical signs of increasing or decreasing edema correlated well with the alterations in the chemical spaces. In three instances there were clinical signs of extracellular fluid volume deficit, and in each instance the chemical spaces were below normal

DISCUSSION

The correlations pointed out in this paper are only gross estimates of change. Since neither of the two variables can be considered the more accurate, two conclusions may be drawn from each correlation, depending upon the weighting of the variables.

The almost unity correlation which exists between weight change and the change in the chemical spaces indicates that:

1. If the chemical spaces were accurate, during the first 1 to 2 weeks

* Crosses represent 7 to 9 day cumulative sodium balance, which has a much larger error.

during the past year with further refined enzymes whose microbiological activities are similar to those previously elucidated.[1]

METHODS AND MATERIALS

We obtained 3 enzymes from the Lederle Laboratories referred to as 7-1162-D-11, 7-1162-A-16 and 7-1162-A-17. These enzymes are from two different aerobic organisms. The fourth, proteinase A, obtained from the Sharp & Dohme Laboratories, has been further concentrated to 28,000 u. over the previously reported material.

These materials were obtained as lyophilized powders and were activated by adding 2 cc. of normal saline. This solution of enzyme was added either to large flasks containing 1000 cc. of normal saline or mixed into a water-soluble methylcellulose or tragacanth jelly base.

If the lesion was extensive, we put multiple catheters in the folds of a three-ply gauze dressing and placed them directly on the eschar. A double layer of petrolatum gauze was put over this and the dressing was held in position by a loosely applied Ace bandage. The enzyme solution was then allowed to flow through the catheters quite rapidly to soak the dry, sterile gauze. Then the rate of flow was controlled at 20 drops per minute.

In the other instance where the jelly base was utilized, the enzyme material was liberally spread over the eschar and a three-ply gauze dressing was soaked in the material and applied over the wound site. This dressing was then sealed off as described above. If possible, the dressing was left undisturbed for 12 hours to obtain maximum exposure of the area to the enzyme activity. The wounds were inspected every 12 hours.

In a number of cases where infection was already present in the wound or heavy contamination was suspected, we cultured the wounds and utilized an appropriate broad spectrum antibiotic by adding it to either the solution or the paste so that a bactericidal concentration was obtained. Routine prophylactic parenteral antibiotics are given concomitantly to each patient undergoing lysis of his eschar.

Whenever possible the enzymes were applied to the eschar 8 days after injury. This allows the patient time for physiologic stabilization and permits the body defenses to demarcate the eschar.

The clinical studies presented here were carried out on the Surgical Services of St. Vincent's Hospital and, at the invitation of the U. S. Navy, at the Newport Naval Hospital under the supervision of Captain John L. Enyart and Commander Donald W. Miller. In all, 18 patients with severe second and third degree burns, 11 patients with varicose or decubitus ulcers and 4 patients with miscellaneous lesions have been studied since our 1953 report. This presentation will be limited to the burn study.

RESULTS

Lederle 7-1162-D-11. This enzyme was extensively used in 2 of the burned patients at the Newport Naval Hospital. In both cases the eschar had been present for 22 days. The eschar, at the time of application of the enzymes, was thick and adherent but many areas had broken down and had been removed by mechanical débridement, leaving behind areas of exudative granulation tissue (Fig. 1).

The enzyme D-11 was first applied to the first patient's right arm as a continuous drip for 17 hours at 7000 u. per cubic centimeter combined with

movement would reflect changes in the true volume of distribution of sodium.

The time sequence plot of the change in the chemical spaces indicates that the prompt resolution of the burn wound edema beginning at about 48 to 72 hours is not followed by a corresponding decrease in the extracellular fluid volume. The reabsorbed fluid is redistributed in the entire extracellular fluid volume, and is slowly lost over a period of two weeks.

CONCLUSIONS

Although absolute accuracy of these spaces has not been determined, the Δ function of sucrose is appropriate in direction and probably in magnitude when judged by clinical signs, weight change, and sodium balance change. From these data it appears that the use of sucrose is preferable to the use of sodium thiosulfate as an estimate of the extracellular fluid volume. The thiosulfate space may be a better estimate of the volume of distribution of sodium than of the extracellular fluid volume

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FURTHER OBSERVATIONS ON THE USE OF PROTEOLYTIC ENZYMES IN THE REMOVAL OF THE BURN ESCHAR*

JAMES F. CONNELL, JR., AND LOUIS M. ROUSSELOT**

At the 1953 meeting of the Surgical Forum of the College we presented our data on the use of 5 proteolytic enzymes then under investigation for the removal of burn eschar Our conclusions on these materials were that they were all remarkably active in vitro, but, with the exception of clostridial filtrate, were not dramatic in animal and clinical trials

In this report we will present, for the most part, our clinical experiences

* From the Department of Surgery, St Vincent's Hospital, New York City. This work was supported (in part) by the Medical Research and Development Board, Office of the Surgeon General, Department of Army, Contract No. DA-49-007-MD-72.

** With the cooperation of Captain John L Enyart (M C , U S N), Director, Newport Naval Hospital, and Commander Donald W. Muller (M C , U S.N), Chief of Surgery, Newport Naval Hospital, Newport, Rhode Island.

room prior to grafting. Our conclusion from this instance, confirmed by a second patient and in vitro and animal studies, is that this enzyme does not lyse the eschar but separates it from the wound at the interface of wound and eschar.

Proteinase A. Six patients with third degree burn wounds were treated

Fig. 3.



Fig. 4.

Fig. 3 After failure of the above enzymes in low concentrations, enzyme 7-1162-D-11 was applied in high concentration for 18 hours Note removal of all eschar just prior to grafting.

Fig 4. Eschar covering right arm and trunk of patient with 65 per cent third degree burn 13 days after injury and just prior to treating right arm with enzyme 7-1162-A-16.

500 mcg. per cubic centimeter of water-soluble Terramycin.* The result was a wetting of the still adherent eschar and removal of all exudate. The wound odor was remarkably improved as compared to the control area where normal saline alone was utilized. This area of the arm was then subjected to 18 hours of proteinase A—112 u. per cubic centimeter plus antibiotic.

Fig. 1.



Fig 2.

Fig 1. Right arm Eschar 22 days after injury has been removed in a number of areas by mechanical débridement The eschar remaining is fairly adherent

Fig 2 After 17 hours of exposure to enzyme 7-1162-D-11 and 18 hours of proteinase A the wound demonstrates removal of exudate but only wetting of eschar.

On inspection the eschar was still adherent in most instances (Fig. 2). In a few spots small islands of eschar were wiped off. The wound was then perfused with normal saline and redressed. For the next 24 hours Lederle 7-1162-D-11 was utilized at 50,000 u. per cubic centimeter, or a sevenfold increase over the first day's solution. The wound was completely free of all eschar, exudate and odor. The view in Figure 3 was taken in the operating

* Supplied through the courtesy of Pfizer Laboratories.

and animal tests indicated that this enzyme was separating rather than lysing the eschar.

Lederle 7-1162-A-16 and 7-1162-A-17. To date, 8 patients with severe burns have been treated with A-16 and 3 with A-17. The trials with A-16 gave evidence of promise. The enzyme in concentrations of 50,000 u. per cubic centimeter was applied in a jelly base to the right arm of a woman with a 65 per cent body surface, third degree burn (Fig. 4). The material was started on the thirteenth day following injury and the first day after admission to our Surgical Service. Note in Figure 5 that the eschar com-



Fig. 7. Note the extensive amount of surgery required to remove only one-third of the eschar from this patient's back.

pletely *separated* after 3 days of exposure to enzyme. Use of this enzyme at the Newport Naval Hospital gave similar results and indicated that this enzyme or enzyme source needed further trials. 7-1162-A-17 was obtained as a next step and to date in 3 patients has demonstrated that the activity has been similar to A-16. However, we have noted some lysis rather than just separation. This is important, as we feel that separation will always be the much slower process.

There were no signs of either local or systemic toxicity with the local use of the various enzyme materials

In conclusion we wish to show that unless we keep up this intensive study of these enzymes, we will have to continue the harrowing and traumatic task of removing the burn eschar in the severely burned patient by surgical débridement (Figures 6 and 7).

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with this enzyme, including the above described instance. Of the remaining 5 patients, 4 were treated on the eighth day after injury and one on the twenty-second day. In concentrations of 115 u. per cubic centimeter no effect but wetting was noted. When the concentration was increased to 1200 u. per cubic centimeter separation of eschar from granulation surface was noted in 36 to 48 hours, the older wounds requiring the less time. In vitro

Fig 5.



Fig. 6.

Fig 5. Complete removal of eschar from right arm after 72 hours of enzyme therapy.
Fig 6. The photograph demonstrates the extensive eschar over posterior trunk of patient described above

SHOCK AND WOUNDS; PLASTIC ANATOMIC CAST

THE EFFECT OF LEVARTERENOL ON RENAL BLOOD FLOW IN DOGS SUBJECTED TO HEMORRHAGIC SHOCK*

JOHN H. FOSTER, HAROLD A. COLLINS, AND H. WILLIAM SCOTT, JR.

In the last few years levarterenol† has been used clinically to maintain the blood pressure in a variety of acute hypotensive states. Of interest is the use of this vasopressor drug in patients who remain hypotensive in spite of adequate, or even excess, whole blood replacement following hemorrhagic shock. Crawford and Haynes,[4] who advocate blood volume studies to determine the adequacy of blood replacement, have found levarterenol helpful in those patients who are hypotensive in spite of apparently adequate whole blood therapy. These authors and others[4, 6, 11-13] have found that after the administration of levarterenol from two to sixty hours many patients were then able to maintain normotensive arterial pressure. In these circumstances it has been felt that changes leading to so-called "irreversible shock" have been avoided by preventing prolonged hypotension

Renal shutdown after prolonged periods of hypotension is a well known and often fatal complication. Renal ischemia is believed to be the prime factor in renal shutdown.[8, 12, 13] Renal blood flow is a function of two factors: loading arterial pressure and renal arterial resistance. Levarterenol apparently increases both factors and in the normal state has a net effect of decreasing renal blood flow in both humans and experimental animals.[1, 2, 9, 14] The net effect of levarterenol on renal blood flow in hypotensive states is unknown.

In the present investigation the effect of levarterenol on renal blood flow has been measured, by a direct method, in anesthetized dogs subjected to hypovolemic shock and "irreversible hemorrhagic shock."

METHODS

Healthy mongrel dogs weighing from 10 to 24 kg. were anesthetized with intravenous sodium pentobarbital (30 mg. per kilogram). Endotracheal intubation was routinely performed to insure an adequate airway. A femoral artery was cannulated for direct determination of mean blood pressure by a mercury manometer. The abdominal cavity was entered through a midline incision, and the vena cava exposed in the region of the renal veins. Any tributaries entering the vena cava within 2 cm. of the renal veins were ligated. Great care was exercised to avoid trauma to the renal pedicles. Tapes were passed around the vena cava 2 cm. above and below the entrance of the renal veins. This segment of the vena cava was partially

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† Nor-epinephrine, l-arterenol, Levophed, furnished courtesy Winthrop-Stearns, Inc., Medical Research Division, New Orleans, Louisiana.

istered until the pressure was stabilized at 100 to 110 mm. Hg and renal blood flows were measured three times at ten minute intervals. These measurements of renal blood flows in animals with "irreversible hemorrhagic shock" were then repeated twice, with and without the administration of levarterenol.

As a method of elucidating the effect of levarterenol on "normal" renal blood flow, the drug was administered to five animals following control determinations. Levarterenol was administered by intravenous drip at a rate sufficient to maintain mean arterial pressure at 150 to 160 mm. Hg.

A third group of animals served as controls for the second hour of hypovolemic shock in the animals of group I. Whereas the animals in group I received levarterenol infusion during the second hour of hypovolemic shock, the drug was omitted during that period in this group. Preliminary steps were exactly as in group I, but no attempt was made to regulate the pressure during the second hour of hypovolemia. Thus a control on the natural course of events was obtained.

RESULTS

Initial or pre-shock renal blood flows ranged from 16.4 to 22.4 ml. per kilogram body weight per minute, with an average of 18.1. The animals with the lower flows were consistently the ones in which a fall in blood pressure occurred incident to the operative trauma and prior to bleeding. There was little variation in the flows in individual animals with repeated measurements in this period.

Group I: The Effects of Levarterenol on Renal Blood Flows of Hypovolemic Shock and Irreversible Hemorrhagic Shock. In the first group of animals hypovolemic shock was associated with a marked decrease in renal blood flow in each instance. These results are summarized in Table 1. After bleeding to a mean arterial pressure of 40 mm. Hg and maintenance at this level for one hour the renal blood flow decreased to a range of 2.2 to 6.4 ml. per kilogram per minute and averaged 3.3 ml. per kilogram per minute. During this one hour period the flow determinations were consistent in almost all animals. For the subsequent one hour period in which arterial pressure was maintained at 100 to 110 mm. Hg by the administration of levarterenol there was an increase in the renal blood flow in all animals. The renal flow ranged from 3.0 to 10.7 ml. per kilogram per minute and averaged 6.04. Thus an increase in renal blood flow of almost 100 per cent resulted from the administration of levarterenol to these dogs in hypovolemic shock (see Fig. 2).

The restoration of blood volume by infusion of all shed blood increased renal blood flow to the range of 5.2 to 22.4 ml. per kilogram per minute with an average of 13.9. In most animals the renal blood flow during this period approximated that of the control period. This was an increase of 300 per cent over the hypovolemic hypotensive values and of 100 per cent over the hypovolemic-levarterenol-normotensive values. In eight out of ten animals in this group "irreversible hemorrhagic shock" developed within one to three hours after infusion of all shed blood. Renal blood flow measurements ranged from 0.7 to 8.0 ml. per kilogram per minute and averaged 3.35 when measured at mean arterial pressure levels of 70 mm. Hg or lower. During thirty minute periods of infusion of levarterenol at a rate sufficient to maintain the arterial pressure at 100 to 110 mm. Hg, the renal flows ranged from

occluded with a curved Potts patent ductus clamp, and a glass cannula (6 mm I.D.) inserted. The cannula was secured with a purse-string suture. Direct collections of renal blood flow were made through rubber tubing leading from the cannula. Tubing and cannulae of various calibers were tested, and a diameter of 6 mm. proved to accommodate flows well in excess of measurements in this study. Figure 1 illustrates the completed preparation.

After a collection was completed the tapes were released and sufficient heparinized saline infused to clear the system (10 to 20 cc.). Mean arterial blood pressure was recorded before and after each collection.

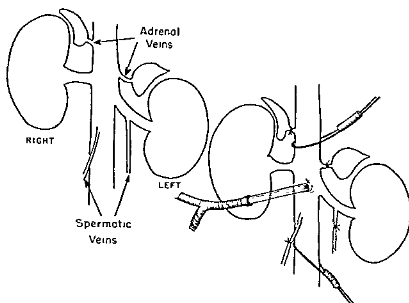


Fig 1. The cannulization of the vena cava for the direct determination of renal blood flow.

In the first group of animals, while still normotensive, renal blood flows were measured at ten minute intervals over a control period of thirty minutes Each animal was then bled until arterial pressure was reduced to 40 mm. Hg. This level was maintained for one hour. Bleeding of 400 to 800 cc. initially and of an additional 150 to 300 cc during the hour served to keep the pressure at 40 mm. Hg. Renal blood flow was measured every fifteen minutes. At the end of an hour a slow drip of normal saline containing 12 mg. levarterenol was started. The drip was regulated to maintain the mean arterial pressure in the range of 100 to 110 mm Hg. Renal blood flows were measured every fifteen minutes during the hour. All shed blood was then infused intravenously over a period of one hour. Renal blood flows were determined every thirty minutes. In eight out of ten animals in this group "irreversible hemorrhagic shock" developed, and during this period renal blood flows were determined three times at ten minute intervals with the mean arterial pressure less than 70 mm Hg. Levarterenol was then admin-

0.8 to 12.9 ml. per kilogram per minute and averaged 4.95. While the differences were small, the renal blood flow was always greater with levarterenol elevation of arterial pressure than without it during this irreversible phase.

Group II: The Effect of Levarterenol on Normal or Pre-Shock Renal Blood Flow. The effect of levarterenol administration on the renal blood flow was assessed in five animals not subjected to hypovolemic shock. The results in these dogs are summarized in Table 2. The flows during the control

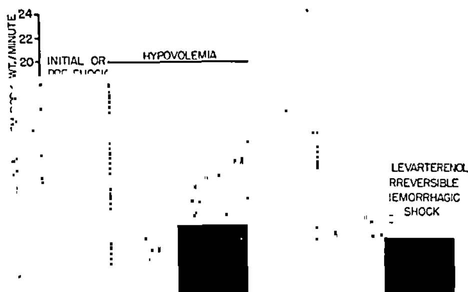


Fig. 2

period ranged from 16.6 to 22.6 ml. per kilogram per minute and averaged 19.4. The infusion of levarterenol at a rate which maintained the mean arterial pressure at 150 to 160 mm. Hg effected a decrease in the renal blood flow to a range of 4.0 to 13 6 ml. per kilogram per minute, or an average of 8 7. The administration of levarterenol to these normovolemic animals thus diminished renal blood flow in excess of 50 per cent.

Group III: The Effect of Duration of Hypovolemia on Renal Blood Flow. In order to evaluate the effect that duration of hypovolemic shock has on

Table 2. Effect of Levarterenol on Normal or Pre-shock Renal Blood Flow

		RENAL BLOOD FLOW IN CC./KG./MIN	
DOG NO.	WEIGHT IN KG.	INITIAL OR PRE-SHOCK	LEVARTERENOL 150-160 MM. HG BLOOD PRESSURE
406	18 4	17.4	10 9
419	14 0	22 2	13 6
421	11 0	18 2	6 2
423	13 3	22.6	9.0
425	12 0	16 6	4 0
Average		19.4	8 7

Table 1. Renal Blood Flows in Hypovolemic Shock and Irreversible Hemorrhagic Shock with and without Administration of Levarterenol

		Renal blood flows in cubic centimeters/kilograms body weight/minute					
			Hypovolemia				
Dog No.	Weight in Kg	Initial or Pre-Shock	40 mm Hg Blood Pressure One Hour	Levarterenol 100 mm Hg Blood Pressure One Hour	Restored Blood Volume	Irreversible Hemorrhagic Shock	Irreversible Hemorrhagic + Levarterenol
322	13.0	22.4	6.4	10.7	22.4	7.8	9.2
323	16.2	20.0	4.5	5.0	7.2	*—	—
324	23.0	16.4	3.1	8.2	6.2	2.0	4.0
325	12.4	17.5	3.9	9.6	18.7	8.0	12.9
330	23.3	16.8	1.4	3.1	16.6	2.6	4.3
353	13.2	19.1	2.7	5.4	7.3	1.5	2.0
384	10.4	16.5	2.3	3.1	17.9	0.7	0.8
406	18.4	17.4	2.2	5.0	5.2	1.7	2.7
392	13.4	16.7	3.2	3.0	16.4	*—	—
421	11.0	18.2	3.0	7.3	21.0	2.5	3.7
Average	—	18.1	3.27	6.04	13.9	3.35	4.95

*Irreversible hemorrhagic shock did not develop

flow to almost zero by raising the arterial pressure to 200 to 250 mm. Hg in each instance.

Renal blood flow is a function of two opposing factors: the loading arterial pressure and the renal arterial resistance. Levarterenol increases the arterial pressure by augmenting peripheral resistance.[9, 12, 13] It increases both factors which influence renal flow. In the normal state, or with a high dosage, it is probable that the increase in renal arterial resistance predominates and renal blood flow decreases.

In hemorrhagic or traumatic shock the decrease in renal blood flow is disproportionate to the fall in cardiac output.[9] This has been interpreted to represent a protective mechanism for redistribution of blood to more vital areas. The evidence suggests that the renal ischemia thus produced plays a major role in the production of lower nephron nephrosis and renal shutdown.[9,] [illegible] levarte[illegible] shock" [illegible] under these circumstances. The state of shock possibly effects maximal or near maximal renal vasoconstriction, thereby limiting levarterenol effect to that of raising loading arterial pressure with an attendant increase in renal blood flow.

The infusion of levarterenol in animals subjected to hypovolemic shock produced a consistent increase in renal flow, which averaged 100 per cent. In no instance was there a decrease in renal circulation. The control studies indicated that levarterenol was responsible for the augmentation of renal blood flow, as passage of time alone had no such effect.

Whole blood transfusion is the therapy of choice in most forms of shock. Its superiority in correcting renal ischemia in these studies is striking. Renal blood flow was returned to control levels in most instances by restoration of blood volume. The increase was 200 per cent greater than that effected by levarterenol administration. Mean arterial pressure was returned to normal levels in every instance.

The onset of "irreversible hemorrhagic shock" was accompanied by a fall in renal blood flow to [illegible] Levarterenol infusion [illegible] mentation in renal flow [illegible] the consistency with which it occurred is noteworthy. Thus in both the hypovolemic and "irreversible hemorrhagic" shock of this study, levarterenol increased renal blood flow in every instance.

SUMMARY

A method for the direct determination of renal blood flow which gives results comparable to those obtained by other methods is described. The effect of administration of levarterenol on the renal blood flow in dogs under a variety of circumstances was measured.

In dogs subjected to hypovolemic shock, levarterenol was found to increase renal blood flow coincident with the increase in systemic mean arterial pressure. The restitution of normal blood volume by whole blood infusion in hypovolemic dogs was more efficacious than levarterenol in restoring renal circulation towards normal levels. The renal blood flow in animals with "irreversible hemorrhagic shock" was slightly, but consistently, augmented by administration of levarterenol.

renal blood flow, a third group of dogs was subjected to the standard shock procedure as in group I animals. With levarterenol omitted during the second hour of hypovolemia the renal blood flows were measured. During the first hour of hypovolemia, when mean arterial pressure was controlled at 40 mm. Hg, the flows ranged from 3.3 to 3.9 ml. per kilogram per minute and averaged 3 7. With levarterenol omitted during the second hour of hypovolemia, renal blood flows ranged from 2.3 to 3.7 ml. per kilogram per minute and averaged 3.1 (Table 3).

Table 3. Effect of Duration of Hypovolemia on Renal Blood Flow (Control Renal Blood Flow Second Hour Hypovolemic Shock)

		RENAL BLOOD FLOW CC./KG /MIN		
DOG NO	WEIGHT IN KG	INITIAL OR PRE-SHOCK	10 MM HG BLOOD PRESSURE ONE HOUR	2ND HOUR PRESSURE UNCONTROLLED
425	12 0	16 6	3 3	3 3
554	14 4	15 3	3 9	2 3
560	11 2	15 5	3 8	3 7
Average		15 8	3.7	3 1

DISCUSSION

The method used for direct measurement of renal blood flow in this study is not original It was used by Blalock in his studies of renal circulation in 1936–37 [7, 8] Cohen and Lillihei[3] have recently measured azygos flow by a similar method The influence of general anesthesia and operative trauma on arterial pressure and renal blood flow are two of its disadvantages, especially in regard to determination of "normal" values for renal flow Blalock, using local anesthesia, measured the renal blood flow directly by vena caval cannulation via jugular vein and obtained values which ranged from 16 0 to 29.8 ml. per kilogram per minute and averaged 21.1.[8] Using indirect methods, Van Slyke[15] obtained average values of 19.3 ml. per kilogram per minute in normal dogs In the present study, pre-shock or "normal" renal blood flows ranged from 16 4 to 22.6 ml. per kilogram per minute and averaged 18 5. The lower "normal" values obtained in this study by direct measurements are probably explicable on the basis of anesthesia and operative trauma. Low pre-shock renal flow was invariably preceded by a fall in arterial pressure incident to the operative procedure Animals which maintained stable blood pressure during operation had renal blood flow values comparable to those obtained by Blalock.

The administration of levarterenol by intravenous drip to produce a desired arterial pressure is at variance with the method used by most investigators who have studied the actions of this drug.[1, 2, 5, 10, 14] Exact dosage and time schedules have more commonly been used to study its pharmacologic effects. However, clinical usage has followed the pattern used in this work.[4, 6, 11-13] Levarterenol was infused rapidly in several animals at various stages of this experimental procedure. It was possible to reduce renal blood

THE EFFECT OF INDUCED HEMORRHAGIC SHOCK ON THE CEREBRAL CIRCULATION AND METABOLISM OF MAN*

HRANT H. STONE, THOMAS N. MACKRELL, BERNARD J. BRANDSTATER, GERALD L. HAIDAK, AND PAUL NEMIR, JR.

The role of the brain in the pathogenesis and perpetuation of the shock syndrome has not been conclusively established, especially in the human patient. Studies measuring the response of the human cerebral circulation and metabolism to the hypotension of acute blood loss have not been made. Recent investigation of cerebral hemodynamics during acute drug-induced hypotension[1] has revealed an effective maintenance of cerebral blood flow by an adequate degree of relaxation of cerebral vascular tone. During the hypotension of hemorrhagic shock, does a similar degree of compensation occur to ensure the maintenance of cerebral blood flow in spite of the added acute reduction in effective circulating blood volume? Selective vasoconstriction, a compensatory phenomenon operating during secondary hemorrhagic shock, serves to shunt blood from less vital areas, such as the extremities and kidney, to more immediately critical regions.[2] The brain certainly should be one such critical region, although this has not been quantitatively substantiated. The present study was undertaken to clarify this question.

This study restricts itself to the early phases of hemorrhagic shock, and results obtained are not necessarily applicable to shock of longer duration. Early shock as seen in the average civilian hospital or in the operating room resembles the situation produced in this study.

METHOD

Eight studies were performed on five professional volunteer subjects, one patient having three studies (T.E.). Each subject was well screened as to physical and mental health, age, and previous illness prior to being admitted to the hospital. A complete history and physical examination, with comprehensive laboratory studies, constituted the work-up of each subject. Three units of whole blood were typed and crossmatched for each study.

The studies were performed in an operating room under strictly controlled conditions. A continuous electrocardiographic recording was made throughout the study. Two 15 gauge needles were inserted into accessible arm veins and connected to infusion sets containing physiologic salt solution. A needle inserted in the femoral artery was attached to a strain gauge manometer and recorder to obtain a continuous blood pressure tracing. Mean pressure was calculated by planimetry.

With the unpremedicated patient in the supine position, a control cerebral blood flow (*C*) determination was performed and calculated according to the original nitrous oxide method.[3] Each subject was then slowly but pro-

* From the Departments of Anesthesiology and Surgery, Graduate School of Medicine, the Harrison Department of Surgical Research and the Hospital of the Graduate School of Medicine, University of Pennsylvania, Philadelphia This study was aided by grants from the Wilson C. Laucks Memorial Foundation, the A. Atwater Kent Fund, and No. H-1282 from the National Heart Institute of the National Institutes of Health, Public Health Service.

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Table 1. The Effect of Acute Hemorrhagic Shock on Cerebral Blood Flow, Cerebral Vascular Resistance and Arterial pCO_2

Subject		Amount of Blood Removed	% of Blood Vol.	Mean Art. Blood Press.			Cerebral Blood Flow			pCO_2			Cerebral Vasc. Resistance		
				C	E	M	C	E	M	C	E	M	C	E	M
1. W.G.	♂ 71 / 176 lbs.	1500 cc.	20	105	43 (59%)	—	41	37	—	38	30	—	2.6	1.2	—
2. T.E.	♂ 24 / 116 lbs.	1500 cc.	25	82	37 (55%)	—	43	28	—	38	23	—	1 8	1 3	—
3 T.E.	♂ 24 / 116 lbs.	1850 cc.	30		45 (50%)	42		21	39		22	35		2 1	1.1
4. R.S.T.	♂ 36 / 154 lbs	2400 cc.	38	104	53 (49%)	56 (16%)	44	36	45	36	19	25	2.3	1 5	1 3
5. C.K.	♂ 45 / 115 lbs.	2200 cc.	37	91	47 (48%)		57	42		36	30		1.4	1.1	
				mm. Hg			cc./100 g./min.			mm. Hg			Resistance Units		

C = Control.
E = Experiment (shock).
M = Morphine.

gressively bled via the femoral artery until the signs and symptoms of hemorrhagic shock appeared. These findings consisted of mental changes (apprehension, confusion, restlessness, irritability, excitement, nausea and vomiting), generalized perspiration; pilomotor changes; cold, moist skin, increase in rate and depth of respiration; and hypotension. A second cerebral blood flow determination (*E*) was made during the hypotensive episode while the signs and symptoms of hemorrhagic shock actively persisted. Stabilization of the blood pressure at shock levels during the second blood flow determination was accomplished by the additional removal of blood as required. In one subject (R S.) a third cerebral blood flow determination (*M*) was made following the intravenous injection of 10 mg. morphine sulfate. Another subject (T.E.) returned two weeks later for a second study. Since control values were previously obtained, shock was immediately induced and a blood flow determination made following the intravenous injection of 8 mg. morphine sulfate (*M*). Arterial and internal jugular blood samples were analyzed for oxygen and carbon dioxide values using the Van Slyke manometric methods.[4] *p*H determinations were made anaerobically in a glass electrode and corrected for temperature by suitable factors.[5] Blood volume determinations in the initial studies were not made to permit more rapid perfection of the experimental technique Realizing the inaccuracies of the method, estimates of blood volume were made by using the ratio of 90 cc. whole blood per kilogram body weight.

RESULTS

Three of the eight studies were not satisfactory because of the inability to stabilize the signs and symptoms of shock long enough to complete the blood flow determination. In the remaining subjects, a fairly constant response pattern was noted. Following the removal of 20 to 38 per cent of the estimated blood volume with a resulting 48 to 59 per cent reduction in mean arterial blood pressure, there was in each instance a decrease in cerebral blood flow in spite of a reduction in cerebral vascular resistance

onset of severe shock symptoms. Following the administration of morphine sulfate (8 and 10 mg., respectively) to two subjects, there was an immediate rise in arterial pCO_2 to normal values, accompanied by a decrease in arterial *p*H. This was followed by an increase in cerebral blood flow to normal values with a further decrease in cerebral vascular tone.

DISCUSSION

The response of the human cerebral circulation to the hypotension of acute hemorrhagic shock varies from that seen following hypotension of different etiology. The ability of the cerebral vessels to dilate adequately and thus maintain the constancy of cerebral blood flow during drug-induced hypotension is conditioned by the intensity of the hypotensive stimulus. More profound decreases in mean arterial blood pressure initiate more complete vascular dilatation. The failure of the cerebral vessels to dilate ade-

as a respiratory depressant, abolished the existing hyperventilation and permitted the accumulation of carbon dioxide. The striking over-all subjective improvement following the use of morphine was not due to a rise in mean arterial blood pressure, for this remained unchanged. The improvement coincided with the restoration of normal arterial pCO_2 levels. It appears therefore that the response of the human cerebral circulation to early hemorrhagic shock is dependent upon the degree of hyperventilation and alkalosis which develops. Severe alkalosis, capable of restricting cerebral

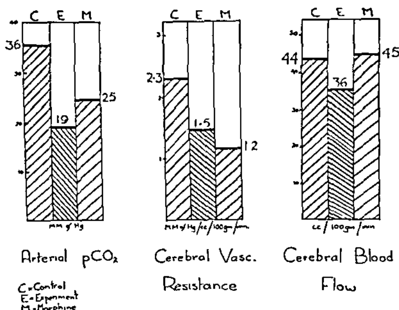


FIG. 2 Patient R S. Alterations in arterial carbon dioxide tension, cerebral vascular resistance and cerebral blood flow produced by acute hemorrhagic shock and intravenous morphine sulphate.

vascular dilatation, may reduce cerebral blood flow by 33 per cent or more[7] and be a prominent factor in the rapid mental deterioration of the patient. While the importance of decreased arterial CO_2 in late hemorrhagic shock has for long been recognized as a compensatory factor,[8] little attention has been given to its significance as an early derangement accompanying shock. Since in civilian practice early shock states are far more common than terminal irreversible stages, further studies of this type are indicated.

SUMMARY AND CONCLUSIONS

1. The effects of acute hemorrhagic shock on cerebral blood flow and cerebral oxygen consumption were determined on five professional volunteer subjects.

2. A decrease in cerebral blood flow occurred in each case accompanied by a rise in cerebral O_2 consumption and an increase in A-V O_2 difference. Cerebral vascular dilatation in response to the hypotension of shock was not adequate.

3. Restriction of cerebral vasodilatation was the result of a respiratory alkalosis secondary to hyperventilation of unknown etiology.

quately during the hypotension of hemorrhagic shock despite the intense stimulus of profound blood pressure reductions must be related to other physiologic derangements which accompany the shock state. The decrease in arterial carbon dioxide tension and the alkalosis which results during hemorrhagic shock constitute a significant difference between that state and hypotension induced by various drugs. Changes in arterial carbon dioxide tension were not observed during drug-induced hypotension. These changes in blood chemistry are probably due to the hyperpnea which so characteristically appeared and persisted during the period of severe shock symptoms. The cause of this hyperventilation is not apparent although it may be reflexly mediated through the pressor receptors or even the chemoreceptors. If it is a pressor receptor reflex, hyperventilation should occur in response to other types of hypotension as well as that produced by acute blood loss. This is

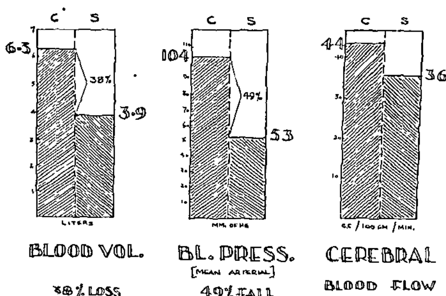


Fig. 1. Patient R.S. The effect of acute blood loss and acute reduction in mean arterial blood pressure on cerebral blood flow.

not the case. An emotional etiologic component might be implicated were it not for the fact that hyperpnea also appears in laboratory animals.

The role of arterial carbon dioxide tension in the control of cerebral vascular tone has been conclusively demonstrated.[6] Carbon dioxide excess (5 to 7 per cent in the inspired air) is one of the most potent cerebral vasodilators available. Conversely, carbon dioxide deficiency will produce a significant increase in cerebral vascular resistance with a reduction in cerebral blood flow It is not clear to what extent the diminished arterial carbon dioxide tensions observed in the present investigations prevented the development of adequate cerebral vasodilatation for the maintenance of cerebral blood flow. That it did play a significant part was demonstrated by the almost immediate return of cerebral blood flow to normal values with spectacular subjective improvement following the administration of 10 mg. of intravenous morphine sulfate (Figs. 1 and 2). Morphine, acting

Healthy adult male and female dogs were used in all experiments reported after adequate control observation periods of 2 to 3 weeks. Using sodium pentobarbital, 25 mg. per kilogram, as the anesthetic agent, the animals were bled 33 per cent of their previously calculated total blood volume as determined by the Evans blue dye technique. The bleeding was accomplished over a 90 minute period with one-fourth of the calculated volume to be removed being withdrawn initially and at 30 minute intervals. Peripheral blood studies including hemoglobin, hematocrit, red blood cell count, white blood cell count, and T-1824 blood volume determinations were performed periodically until control levels were again resumed. No therapy, dietary or otherwise, was administered during this study period. A total of 20 dogs was studied.

Irradiation Alone. The roentgen ray apparatus used for whole body irradiation was a 1000 KVP tube. Irradiation factors were as follows: F.S.D., 200 cm.; filtration, 2 mm. beryllium plus 1 mm. Al; dose rate, 17 r per minute; field, whole body; h.v.l., 12 cm. of tissue or 1.3 mm. Pb; effective wave length, 200 Kev. Dose in free air or within Masonite phantoms was measured with Victoreen 25 r thimble chambers. Dogs were exposed on each side a total of one-half the desired amount. It required approximately 6.5 minutes to administer 100 r total body irradiation as measured in free air, and 13.0 minutes to administer 200 r.[2]

The whole body irradiations were carried out with the animals under sodium pentobarbital anesthesia. A total of 20 dogs received 100 r whole body irradiation alone, and a total of 20 dogs received 200 r whole body irradiation alone. In the postirradiation period studies were made periodically until control levels were again resumed. No therapy of any form was given during the observation period.

Combined Hemorrhage and Irradiation. In this group a total of 10 dogs received 100 r of whole body irradiation followed immediately by hemorrhage of 33 per cent of their blood volume over a 90 minute period. A total of 20 dogs received 200 r of whole body irradiation followed by a 33 per cent hemorrhage. Peripheral blood and blood volume studies were carried out until recovery to control levels.

Group II: Abdominal Surgery with Irradiation. *Combined Intestinal Resection and Irradiation.* Whole body irradiation of 100 r and 200 r was administered to 10 dogs in each category and followed 4 to 48 hours later by intestinal resection and anastomosis. No preparation of the intestinal tract was carried out prior to or following surgery. One foot of ileum was resected at a distance of one foot from the ileocecal valve. Intestinal contents were allowed to spill freely into the peritoneal cavity. An open type end-to-end anastomosis, using 4-0 chromic catgut as the inner through-and-through suture and interrupted Lembert sutures of 4-0 cotton as the seromuscular layer, was carried out. Postoperative blood studies were followed until control le [illegible]

Combi [illegible] al of five dogs were subjected to [illegible] 4 to 48 hours by splenectomy. Blood studies previously described were carried out until control levels were reached.

RESULTS

Group I: Hemorrhage with and without Irradiation. There was no mor-

4. Intravenous injections of morphine sulfate by depressing respiration increased arterial CO_2 tension to normal levels and restored cerebral blood flow. Marked subjective improvement occurred with the return of cerebral blood flow to normal values.

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THE INFLUENCE OF THE ADDITION OF SUBLETHAL IRRADIATION TO HEMORRHAGE AND ABDOMINAL INJURY IN DOGS*

JAMES W. BROOKS, RAY C. WILLIAMS, FREDERICK H. SCHMIDT, WILLIAM T. HAM, AND B. W. HAYNES, JR.

It is likely that a number of persons exposed to a thermonuclear explosion will suffer a combined type of injury involving whole body ionizing irradiation combined with various degrees of trauma which may or may not require surgical intervention. Studies have previously been reported from this laboratory dealing with the effects produced by one form of trauma to be expected in the event of such explosions, namely thermal injury combined with whole body irradiation [1] It was demonstrated that in such an injury a significant increase in mortality followed the combination of a 20 per cent deep second degree burn with 100 r of whole body irradiation Both of these injuries alone are non-lethal. The present study deals with the effect on mortality and recovery of the addition of whole body irradiation to acute blood loss and abdominal surgery in the experimental animal.

PLAN OF STUDY

Group I: Hemorrhage with and without Irradiation. *Hemorrhage Alone.*

* From the Surgical Research Laboratories, Department of Surgery and Department of Biophysics, Medical College of Virginia, Richmond.

eighth days. This was a gradual loss with gradual regain which paralleled the appetite loss and general poor appearance of these dogs during this period.

Group II: Abdominal Surgery with Irradiation. In the 10 dogs receiving 100 r whole body irradiation and intestinal resection and anastomosis there was no mortality. Neither was there any mortality in the group receiving 200 r prior to intestinal resection and anastomosis. In both groups the animals were sacrificed at six weeks, having returned to control levels in all except the white blood count, which was 20 to 30 per cent below control levels, but having shown a steady rise to that level. Autopsies on all animals showed an anastomosis which was intact, having a good lumen, and with no evidence of partial obstruction in the proximal bowel. Adhesions in the

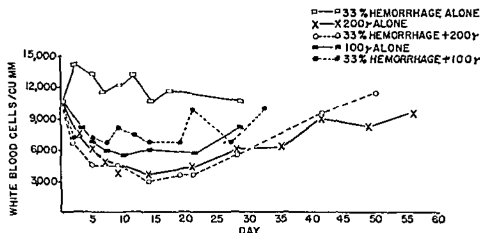


Fig. 2. Diagrammatic illustration of the trend followed by the peripheral white blood cell counts in animal subjected to hemorrhage alone and in combination with whole body irradiation.

abdominal cavity were minimal and of a thin, filmy nature. There was no evidence of local or general peritonitis. The wounds in all dogs healed per primam and sutures were removed from the skin on the seventh day after operation.

In 5 animals who had 100 r of whole body irradiation followed by splenectomy there was one death due to wound evisceration. All other animals recovered without complication in 6 to 7 weeks, with their blood picture following the course previously described.

The lack of any serious difficulty with wound disruption and infection in the series of dogs subjected to surgery is, at least partly, explained on the fact that the healing process is to a considerable extent completed by the time the maximum peripheral blood depression takes place as a result of whole body irradiation. This was not the case in the previously reported burn experiments where the severe wound infection and maximum radiation effects coincide to result in a high mortality from overwhelming septicemia.

SUMMARY

Sublethal trauma, including hemorrhage of one-third of the circulating blood volume, intestinal resection, and splenectomy complicated by sub-

tality in the 33 per cent hemorrhage series alone, in the 100 r and 200 r series alone, or in the 100 r and 200 r series when combined with a 33 per cent hemorrhage. The recovery to normal levels of hemoglobin, hematocrit, red blood cell count, and total red cell mass was twice as long when irradiations were combined with the 33 per cent hemorrhage as with the hemorrhage series alone. Figure 1 illustrates the average response of the hemoglobin in the various groups being considered.

The white blood cell count varied following hemorrhage, showing fluctuations within normal limits. However, when 100 r whole body irradiation was added to hemorrhage a severe leukopenia developed, and the white cell count gradually rose to normal levels in 7 to 8 weeks.

The differential counts on the peripheral blood showed a marked drop in lymphocytes early in the postirradiation phase with gradual replenishment up to the fourteenth day, after which a normal differential count was ob-

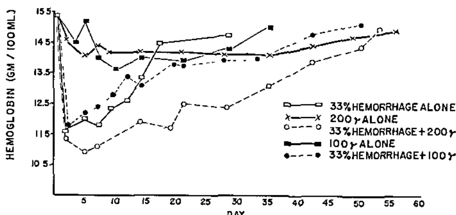


Fig 1 Diagrammatic illustration of the hemoglobin following 33 per cent hemorrhage alone and when in combination with whole body irradiation The red blood cell counts and the red cell mass as determined by blood volume studies followed the same general trend

served Bands and early forms of the polymorpholeukocytes were absent in the irradiated dogs until the twenty-first day after irradiation or later

The same recovery rate was found in the irradiated animal not subjected to external hemorrhage. This severe leukopenia proved most troublesome in our previous studies on burns complicated by whole body irradiation. When a burn wound infection is superimposed on the leukopenia phase, the org

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production occurs but hemorrhage of the quantity studied does not lower body resistance sufficiently to permit significant invasion of microorganisms and cause the development of infection at any one site. Figure 2 summarizes the leukocyte response

There were no significant changes in the animals' body temperatures The plasma proteins remained at normal levels throughout.

Only in the hemorrhage series combined with 200 r whole body irradiation did the weight show significant changes. In these studies it dropped to an average low of 15 to 18 per cent around the fourteenth to twenty-

out while the blood pressure was maintained with a vasopressor agent and for short periods while the vasopressor agent was withheld.

Renal function was determined using inulin clearance as a measure of glomerular filtration rate (GFR) and low concentrations (2 to 4 mg. per cent) of para-aminohippurate to measure renal plasma flow (RPF) employing methods and techniques previously described.[5] Figures listed in the table for these studies are averages of two to three 10 minute collecting periods. Mean arterial blood pressure was calculated by adding one-third of the pulse pressure to the diastolic pressure. The vasopressor agent and rate of administration are listed in Table 1.

RESULTS

During hemorrhagic shock a severe depression in renal function was seen with a high renal vascular resistance. When norepinephrine was administered the increase in renal blood flow far exceeded the elevation in blood pressure, resulting in a striking diminution in renal vascular resistance. Those patients in moderate hemorrhagic shock (R.D., W.H., E.B., and V.S.) responded to norepinephrine by an elevation in renal blood flow to normal or high normal values. Two patients showed only moderate improvement in renal blood flow and are examples of more severe hemorrhagic (M.C.) and traumatic (L.M.) shock. Improvement in glomerular filtration rate in the group with hemorrhagic shock during vasopressor administration paralleled the improvement in renal blood flow. The degree of return in renal function following transfusion appeared to depend on the adequacy of the blood volume replacement.

In the normal volume shock group improvement in renal blood flow and glomerular filtration rate regularly followed the return of blood pressure to normal with norepinephrine. The improvement in renal function in this group was limited and was not as spectacular as in the patients with moderate blood loss shock, probably owing to the poor general condition of these patients.

DISCUSSION

The data demonstrate a paradoxical action of vasopressor agents with respect to the kidneys. While such agents produce renal vasoconstriction in normal subjects, they effect a reduction in renal vascular resistance during hypotensive states. This is apparently explained by a differential vasoconstrictor response in the kidney as compared to the general vascular bed. The return in renal blood flow to normal during norepinephrine administration to patients with moderate hemorrhagic shock was surprising in light of previous experimental work in dogs.[6, 7] The renal blood flow in these animals with induced hemorrhagic shock never completely returned to control values when norepinephrine was given. Only minimal improvement in renal function can be expected in severe hemorrhagic and traumatic shock when vasopressor agents are used. In such cases, as in those of normal volume shock where large amounts of the drug are needed to maintain blood pressure, the relative renal vasoconstrictor potentialities of these agents are realized. When more than 40 to 50 micrograms per minute of norepinephrine was needed to support the blood pressure, little improvement in renal function was observed.

Vasoconstrictor agents are not a substitute for blood volume replacement

lethal whole body irradiation in the experimental animal shows a much longer recovery period than when no irradiation is present. However, the over-all mortality is not greatly, if at all, altered by addition of 100 r whole body irradiation. When infection plays a role in the traumatic process complicated by whole body irradiation, such as thermal burns, the mortality is

ciated with failure of leukocytic response.

It would appear that the combination of hemorrhage, abdominal trauma, and irradiation is less lethal than that of thermal burns plus irradiation.

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RENAL FUNCTIONAL RESPONSE TO VASOPRESSOR AGENTS IN SHOCK*

GEORGE C. MORRIS, JR., JOHN H. MOYER, AND H. LISTON BEAZLEY

The use of vasopressor agents in the treatment of shock continues to be a controversial issue.[1] This study was designed to determine what effects norepinephrine has on renal function during shock in man. In normal subjects vasopressor agents produce renal vasoconstriction[2] If vasopressor agents promote additional renal ischemia during shock, their use could be injurious to the kidney. Recent work indicates increased cerebral and renal *blood flow in response to vasopressor agents given during the hypotension* resulting from ganglionic blocking agents.[3, 4] Obviously the question has arisen as to whether the same benefits can be expected when vasopressor agents are given as treatment for hemorrhagic hypotension and shock due to causes other than hemorrhage.

METHODS

Six patients with hemorrhagic shock and six patients with normal volume shock of varied origin were studied In the hemorrhagic shock group, renal function determinations were carried out during shock, for a brief period while normotension was maintained with norepinephrine or Aramine, and following restitution of the blood volume deficit with whole blood. Prior to the studies 1000 cc of 5 per cent glucose in distilled water was administered intravenously to insure satisfactory hydration for urine formation. In the normal volume shock group renal function determinations were carried

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in hemorrhagic shock or for definitive treatment in normal volume shock. They can be expected to offer protection from renal ischemia while blood is being made available or while diagnostic and specific measures are being taken in the case of normal volume shock.

SUMMARY AND CONCLUSIONS

1. Renal function studies have been made in patients suffering with clinical shock due to various causes. Renal function was determined during

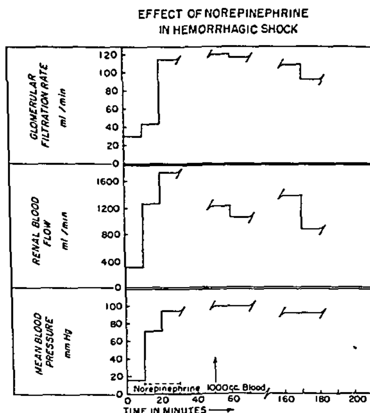


Fig. 1. Graphic representation of the renal functional response to norepinephrine and to blood in a 31 year old male with hemorrhagic shock from extensive lacerations. Renal function was restored to normal levels with norepinephrine. Comparable values were obtained after transfusion with 1000 cc blood.

shock, during norepinephrine-induced normotension and after blood volume replacement in those patients with hemorrhagic shock.

2. Norepinephrine administration to normotensive human subjects increases renal vascular resistance and depresses renal blood flow. When given in shock, renal vascular resistance is reduced and renal blood flow increased. This ambivalent action of norepinephrine is thought to result from a differential vasoconstriction within the kidney by contrast to the remainder of the circulatory bed. The elevation in blood pressure incident to the administration of norepinephrine to patients in shock increases general peripheral vascular resistance but the lesser renal vasoconstrictor effect permits an increased renal blood flow and glomerular filtration rate.

3. Norepinephrine improves renal function in all types of clinical shock. In moderate hemorrhagic shock norepinephrine may return renal function

Table 1. Summary of Clinical Data on Patients Treated

PATIENT	AGE	ETIOLOGY OF SHOCK	PREVIOUS THERAPY	DOSE OF NOREPINEPHRINE IN MICROGRAMS / MIN.	CLINICAL RESULTS
			SHOCK DUE TO BLOOD LOSS (HEMORRHAGIC SHOCK)		
R D	31	Blood loss	1200 cc 5% gluc in H_2O	20	Recovered
W H.	38	Blood loss	1000 cc 5% gluc in H_2O	32	Recovered
M.C	45	Blood loss	500 cc 5% gluc in H_2O	2000 (Aramine)*	Subtotal gastrectomy, 15,000 cc. blood; cardiac arrest, resuscitated, died next day
E B	36	Blood loss	1000 cc 5% gluc in H_2O	18	Recovered
L.M.	52	Trauma	500 cc. 5% gluc in H_2O	30	Recovered
V S.	44	Bleeding esophageal varices	Esophageal balloon inserted just prior to study	25	Developed hepatic failure and coma Died in 48 hours
			NON-HEMORRHAGIC SHOCK OF VARIED ETIOLOGY		
G W.	70	Bile peritonitis	Simple closure of gastric ulcer 48 hours previously	12	Expired in 1 days
L H	29	Intrauterine Lysol	500 cc. blood, D O C A, cortisone, gluc. in H_2O	84	Recovered after stormy course
J N.	70	Multiple bony metastases, terminal carcinoma	Orchidectomy T U R. 10 days before	22	Expired in 4 days
V W.	24	Postpartum triplets	1000 cc blood, I V fluids	17	Recovered
G H	51	Hepatic coma	1000 cc. blood, I.V fluids, Gantrisin	96	Expired following day
J M	44	Electrolyte imbalance, K=2 2 mEq /L.	Nitrogen mustard, blood and I V crystalloids	19	Recovered from shock in 24 hours after K corrected

*Aramine administered instead of norepinephrine in this instance

to normal. Partial restoration of renal function may be effected with the drug in severe hemorrhagic shock, but complete restoration occurs only if the blood volume is increased toward normal.

4. It is suggested that norepinephrine may be useful in protecting the kidneys in certain cases of hemorrhagic and traumatic shock while awaiting blood volume replacement.

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EVALUATION OF A STANDARD TILT TEST FOR ESTIMATION OF BLOOD VOLUME DEFICIENCY*

CURTIS P. ARTZ

Not infrequently a patient is admitted to the hospital with an appreciable blood loss and still he appears to be in good condition. Such a patient has been able to compensate for his blood volume deficiency by vasoconstriction. From the usual clinical signs, he appears to be able to withstand operation, however, when his compensatory ability is abolished by anesthesia, he may have a blood volume deficiency of 1000 cc. or more and his blood pressure may fall. When further blood loss occurs during operation, the resulting oligemia may be severe.

In the management of large numbers of casualties in a forward surgical hospital in Korea, it was frequently observed that movement of the patient to x-ray abolished compensatory mechanisms. Because of this, it was believed that some type of standard postural change might be of value in determining the circulatory status of an injured patient.

It was the purpose of this study to evaluate the use of a standard tilting procedure in order to estimate blood volume deficiency and adequacy of preparation for operation.

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Table 2. Renal Hemodynamic Response to Vasopressor Agents During Shock

Patient	Mean Blood Pressure mm Hg					Renal Blood Flow ml/min					Renal Vascular Resistance*					Glomerular Filtration Rate, ml/min					Hematocrit				
	C	V_1	V_2	B_1	B_2	C	V_1	V_2	B_1	B_2	C	V_1	V_2	B_1	B_2	C	V_1	V_2	B_1	B_2	C	V_1	V_2	B_1	B_2
HEMORRHAGIC SHOCK																									
R D, 280	17	72	93	98	91	301	1239	1717	1159	1125	06	06	05	09	08	29	47	115	119	103	36	36	35	41	41
W H, 281	64	86	89	91F	103	f	720	1021	333	+	—	12	09	27	+	3	114	92	67F	155	30	30	30	35	33
M C, 282	60	73	—	81F	—	121	389	—	547	—	49	19	—	15	—	10	68	—	82F	—	26	26	—	35	—
E B, 283	50	96	—	99	—	126	1286	—	1838	—	40	08	—	05	—	11	86	—	73	—	39	40	—	47	—
L M, 285	43	76	85	70F	83	f	340	680	864	911	f	22	13	08	10	f	5	16	23F	119	25	25	28	28	38
V S, 286 (A)	42	52	74	77	—	258	1291	1627	746	—	16	04	05	10	—	f	66	94	18	—	38	33	33	39	—
(B)	58	67	—	77	—	330	679	—	746	—	18	10	—	10	—	31	53	—	18	—	38	33	—	39	—
NONHEMORRHAGIC SHOCK OF VARIED ETIOLOGY																									
G W, 287	83	—	101		—	119	—	274		—	70	—	37		—	5	—	15		—	50	—	50		—
L H, 289	57	—	98		83	69	—	104		483	83	—	94		15	12	—	25		87	40	—	40		30
J N, 290	67	—	89		—	317	—	388		—	19	—	23		—	33	—	38		—	28	—	25		—
V W, 291	f	86	112		97	—	105	168		255	—	82	67		38	—	10	7		44	—	50	50		24
G H, 292	37	74	92		—	24	59	35		—	1 50	1 20	2 60		—	5	11	12		—	27	27	27		—
J M, 293	37	69	92		—	4	—	159		—	9 20	—	20		—	3	31	69		—	40	—	42		—

*Renal Vascular Resistance = $\frac{\text{Mean Blood Pressure}}{\text{Renal Blood Flow}}$

C = Control during shock
V_1 = Partial restoration of blood pressure with a vasopressor agent (norepinephrine, save M C and V S (A), where Aramine used)
V_2 = Complete restoration of blood pressure with a vasopressor agent
B_1 = Immediately after transfusion of blood
B_2 = Follow-up study—R D 2 hours after B_1, W H 5 days after B_1, L M 24 hours after B_1, L H 25 days after V_2, V W 6 days after V_2
F = Insufficient blood replacement at time of study.
+ = Sulfa drug therapy interfered with PAHA determinations
f = Undeterminable due to extremely low urine or PAHA clearance

Seven patients were selected for specific study. All patients showed a significant fall in blood pressure after the initial tilt, but no significant deterioration was observed following adequate transfusion. All the patients who withstood tilting for 10 minutes or more did not experience hypotension at the time of anesthesia. The following brief summaries illustrate salient points.

Patient No. 1 (Fig. 2). An American soldier was admitted with a blood pressure of 120/80, pulse 78, following small penetrating wounds of the upper quadrant of the abdomen and buttocks. His condition appeared excellent. When tilted for seven minutes, he became nauseated and pale, and

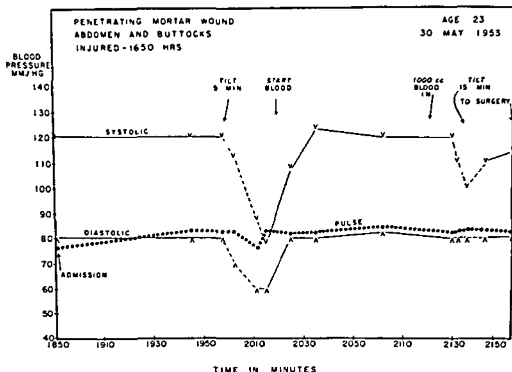


Fig. 2. Chart of patient No. 1. This patient's blood pressure was normal, but his condition deteriorated when he was tilted. Later, after infusion of 1000 cc. of blood, he withstood tilting well.

sweat broke out on his forehead. His blood pressure fell to 78/60. Following administration of 1000 cc. of blood, his blood pressure remained stable after tilting. He experienced no difficulty during splenectomy and débridement.

Patient No. 2 (Fig. 3). An American soldier received perforations of the colon, pancreas, kidney, and spleen from small arms fire. He was admitted with a blood pressure of 120/60, pulse 84; but when tilted, his blood pressure fell to 70/30 within three minutes. After each 500 cc. of blood, the tilt test was performed. At each tilt, blood pressure fell until he had received 1250 cc. of blood, at which time there was no particular deterioration in his condition. He withstood the extended operative procedure well, in spite of a tremendous blood loss. In the first 24 hours after injury, the total amount of blood received was 14 pints. He experienced no difficulty until seven days postoperatively, when he developed a severe pancreatitis and

METHODS

A simple tilt table was devised (Fig. 1). The table was made of wood and had a platform of convenient size to hold a litter. The platform was suspended with the fulcrum in the center. This type of table enabled a litter patient to be placed on the platform so that his head could be raised or lowered. The degree of tilt could be adjusted as desired.

All patients studied were young, healthy males who had been injured in combat. When a patient's vital signs were approximately normal, he was tilted for varying periods of time with his body elevated to a 30 degree angle position, head up. If his blood pressure fell and his condition deteriorated on tilting, it was believed that appreciable blood volume deficiency was

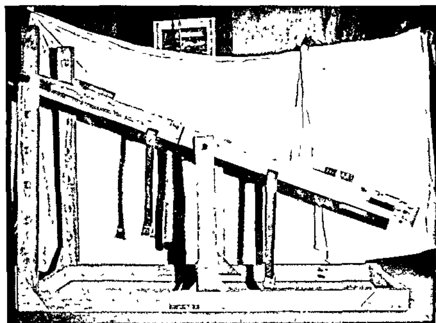


Fig 1. A tilt table made of wood by carpenters in Korea The platform is on a fulcrum The patient on the litter is placed on the platform

present All patients were tilted at this same angle, head up. Patients who were put on the tilt table were examined carefully to make sure that hemorrhage had ceased and that physiologic derangements other than blood volume deficiency had been corrected. The duration of the tilt and the degree of deterioration of the patient's condition was correlated with the amount of blood required to stabilize the patient.

RESULTS

Several patients admitted to the preoperative section of the forward surgical hospital were placed on the tilt table and their response to tilting was observed. All patients who showed definite deterioration when placed in the tilt position for several minutes remained relatively stable when tilted *after* adequate transfusion.

The tilting of a patient aided the surgeon considerably in determining the need for blood.

was tilted for 15 minutes. Blood pressure remained stable. After receiving 75 mg. of Demerol intravenously, he was tilted again for 10 minutes. On tilting, no change in vital signs was observed. He withstood without difficulty a rather extensive débridement of the multiple grenade wounds of the thigh.

Patient No. 6. A Korean soldier was admitted with a blood pressure of 70/40, pulse 100, with a land-mine wound of the right lower leg and a penetrating wound of the left upper quadrant of the abdomen from which 24 inches of small bowel protruded. He was pale, and there was a clinical impression of severe oligemia. He received 1000 cc. of blood during the next hour, and his blood pressure rose to 92/30. On tilting, his blood pressure fell precipitously and his pulse became irregular. He then received a second liter of blood and his blood pressure rose to 124/90, pulse 112. After a 14 minute tilt, no change occurred in blood pressure or pulse. Following an additional infusion of 500 cc. of blood, a prolonged operative procedure was carried out without difficulty. His good response to anesthesia and the operative procedure indicated that he was well resuscitated.

Patient No. 7. A Korean soldier was admitted to the hospital three hours after receiving mortar fragment wounds of the abdomen, shoulder, open comminuted fracture of the right femur and below-knee traumatic amputation of the right leg. He had an unobtainable blood pressure. Three intravenous infusions and one intra-arterial transfusion were started. After infusing nine pints of blood, his blood pressure rose to 128/90. It was difficult to determine whether or not this patient had received sufficient blood to withstand operation. He was tilted for 10 minutes, and there was no change in blood pressure or pulse rate. He was given 3000 cc. of blood and 1000 cc. of dextran during the prolonged operative procedure. He experienced no difficulty during operation and recovered.

This very severely wounded patient required a vast quantity of blood for resuscitation. The use of the tilt test was of value in determining when adequate blood replacement had been carried out.

DISCUSSION

Observations in this study show that a patient whose blood pressure was within normal limits, but who apparently had lost a fair amount of blood, would have a fall in blood pressure upon tilting his body to a 30 degree angle, head up Immediately following adequate blood replacement, however, he was able to tolerate the same change in body position without fall in blood pressure.

It is generally believed that, following blood loss, compensation by vasoconstriction is mediated through the sympathetic nervous system. When the body of a hypovolemic patient is tilted, decreased blood to the brain makes for cerebral anoxia. With the loss of central nervous system control, sympathetic activity disappears and the compensatory mechanism is abolished.

Determination of blood volume before operation would be ideal if time, equipment, and space would permit. In most hospitals, this is not only impracticable but almost impossible as a routine procedure. On the other hand, the tilt test is a simple procedure. It can be utilized rapidly, without discomfort to the patient and without critical equipment. Although no blood volume determinations were performed in correlation with responses to the

peritonitis and died. During his preoperative resuscitation, this patient was tilted at various intervals and, in each instance, his response appeared to give a good indication as to the status of his circulation.

Patient No. 3. A Korean soldier was admitted to the hospital with a blood pressure of 120/80, pulse 84, following a penetrating wound of the left chest. He appeared to be in excellent condition. When he was tilted for five minutes, his blood pressure fell and cold perspiration broke out on his face. Following infusion of 1000 cc. of blood, his blood pressure remained stable at 124/76 during a 13 minute tilt. He had no difficulty during the closure of the sucking wound of the chest.

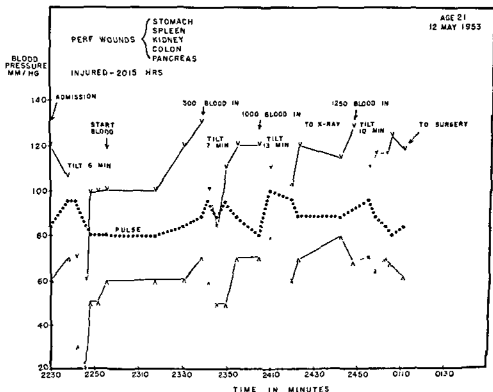


Fig 3 Chart of patient No 2 The response to tilting during resuscitation was in direct relation to the amount of blood given

Patient No. 4. A Korean soldier was admitted with a blood pressure of 120/68, pulse 88, a few hours after receiving a perforating wound of the upper abdomen. After tilting, his blood pressure fell to 88/57, pulse 120. His blood pressure rose to 120/60 when he was lowered to a horizontal position. After infusion of 1000 cc of blood, he was tilted again. Following a 15 minute tilt, there was no change in his blood pressure. At operation there were perforations of the liver, duodenum, and transverse colon. He withstood the operative procedure well

Patient No. 5. An American soldier was admitted with a blood pressure of 110/60 following a grenade wound of the right thigh. After a 5 minute tilt, his blood pressure fell to 80/50, pulse 86. He was pale; and he complained of being sleepy and thirsty. He was shifted to a level position and given 1000 cc. of blood rapidly. His blood pressure rose to 120/80, and he

EVALUATION OF BLOOD LOSS FROM A STANDARDIZED WOUND AFTER DEXTRAN*

ALVIN W. BRONWELL, CURTIS P. ARTZ, AND YOSHIO SAKO

There have been recent reports that the intravenous infusion of dextran may prolong the bleeding time. Carbone and co-workers,[1] in a study of the metabolic effects of dextran, observed two patients who showed definite abnormal bleeding tendencies after infusion of dextran. One of these patients was given 6000 cc. of dextran during a period of six days. He had repeated epistaxis followed by severe bleeding from a duodenal ulcer. The other patient had a severe hemorrhage when a sebaceous cyst was incised after the infusion of 14,000 cc. of dextran during a period of 14 days. In addition, in a group of 50 volunteers given 1000 cc. of dextran, 14 had bleeding times in excess of 10 minutes and two of these had bleeding times of more than three hours.[1] Furth and co-workers[2] studied several brands of dextran in 121 volunteers and found prolonged bleeding times in three subjects, all of whom received the same lot of dextran.†

Experiences with more than 2000 casualties in Korea[3] who received dextran‡ did not suggest that dextran caused increased bleeding.

Observations on patients at Walter Reed Army Hospital, who were given dextran prior to hernia repair and revision of amputation stump, revealed no evidence of increased bleeding.[4]

In order to better assess these isolated but significant observations, this study was designed to observe bleeding time and to measure blood loss in a standardized surgical wound following the infusion of dextran.

METHODS

Patients hospitalized for treatment of burns were given dextran and bleeding times were determined pre-infusion, post-infusion, and at 4, 8, 12, and 24 hour intervals thereafter. Bleeding times were performed as follows: the ear lobe was cleansed with alcohol; a glass slide was placed behind the lobe; and the ear was pierced with a No. 11 Bard-Parker blade until it came in contact with the glass slide. Blood was allowed to drop from the ear and be absorbed on a gauze sponge. The wound was not touched by the sponge. Bleeding time was that time interval from the moment the ear was pierced until the bleeding stopped.

Several days later, dextran was infused again and at the time interval that the longest bleeding time was previously observed, a skin graft was taken. In all instances, one drum of skin (0.014 inch in thickness) was removed with the same Reese dermatome by the same surgeon. After the removal of the skin, the wound was covered with a layer of fine-mesh gauze. A previously weighed laparotomy pad was placed over the fine-mesh gauze and left in place for 10 minutes. It was then removed and a second weighed pad was applied for an additional 10 minutes. When the bleeding was profuse, it was necessary to use several pads. In all instances, the measure-

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† Lot No 13301A Laros, average molecular size 75,000.

‡ Two lots, average molecular size 43,000 and 48,000.

tilt test, there was clinical evidence during the operative and postoperative period of the patient's stable response to a 10 minute tilting. This proved to be a good indication of adequate replacement.

These observations are in agreement with those of Green and Metheny,[1] who studied estimation of acute blood loss by the tilt test. They felt that in the absence of a syncopal reaction, an increase in cardiac rate less than 25 beats per minute on tilting indicated either a negligible or a compensated acute blood loss. Transfusion was not required unless an increase in cardiac rate of 30 beats or more per minute was observed on tilting. This suggested a blood volume deficiency of 9 to 14 cc. per kilogram and a probable transfusion requirement of 1000 cc. of blood. These investigators felt that the occurrence of a syncopal reaction to tilting suggested a probable blood volume deficiency of 1500 cc of blood.

Duncan, Sarnoff, and Rhode[2] studied the effect of postural change in 30 patients with varying degrees of injury They noticed that less severely injured patients who were placed in a head-up position experienced a state of collapse. They believed that this was due to vasodilation and pooling of blood in dependent areas and resultant cerebral anemia.

One of the most perplexing problems in patients who have experienced varying degrees of blood loss is the determination of adequacy of replacement. From the size and character of a wound, experienced surgeons can estimate the approximate amount of blood required before surgery can be attempted. Great difficulty is encountered, however, in determining the adequacy of resuscitation in the very severely wounded patient. Without adequate knowledge of the amount of blood lost, it is difficult to determine the state of resuscitation *after* a patient has received six or seven pints of blood Tilting the body of a patient at a 30 degree angle, with head up, seems to be a test of additional value in determining the adequacy of the circulation

SUMMARY

Several wounded patients whose circulatory status was difficult to determine by clinical observation were placed on a tilt table and their bodies were tilted to a 30 degree angle, head up. If the blood pressure fell and the patient showed other signs of deterioration, it was believed that further transfusions were required prior to operation. When the patient's condition remained stable during a 10 minute tilt, it was believed that adequate replacement therapy had been carried out. Although the experience with the tilt table has not been extensive enough to formulate definite policies concerning its routine use, careful studies in several patients suggest that the response to tilting offers additional criteria for determining the status of the circulation and adequacy of replacement therapy.

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Table 1 shows the bleeding times and blood loss after infusing 19 patients with dextran and serum albumin. In none of the eight patients who received 1000 cc. of dextran was the bleeding time significantly prolonged, and there was no increase in blood loss.

In patients 9 through 15, bleeding times were determined after infusing 1000 cc. of dextran, while blood loss was determined after infusing 2000 cc. There was no significant increase in bleeding time or blood loss except in three patients. Twenty-four hours after infusion of dextran, patient 12 had a [illegible] seconds, but no increase in blood loss at [illegible] r of dextran was given to patient 14, the [illegible] minutes and 25 seconds. He had a significant increase in blood loss at operation. Patient 15 had no increase in bleeding time after infusion of 1000 cc. of dextran, but he had a significant increase in blood loss at operation after 2000 cc. The amount of blood loss

Table 2. Bleeding Times after Infusing Various Types and Amounts of Dextran (Patient 15)

AMOUNT AND TYPE OF DEXTRAN	INITIAL INFUSION (MIN & SEC.)	POST-INFUSION (MIN. & SEC.)	HOURS AFTER INFUSION				
			4	8	16	24	48
1000 cc *	1′ 50″	5′ 15″	7′ 15″	3′ 50″	6′ 15″	7′ 57″	
2000 cc *	1′ 30″	12′ 0″	60′†			31′ 35″	2′ 20″
2000 cc. small-molecular†	7′ 25″	1′ 51″	4′ 21″	1′ 0″	3′ 50″	1′ 20″	
2000 cc. large-molecular‡	3′ 23″	6′ 8″	60′†				

*Lot No. 13301A Laros, average molecular size 75,000.
†Special fractionated C.S C dextran, fraction 3, average molecular size 51,300.
‡Special fractionated C.S C dextran, fraction 4, average molecular size 91,700.

recorded in patients 14 and 15 does not represent the entire amount lost, inasmuch as these patients continued to ooze from the donor wound for 24 hours.

Patients 16, 18, and 19 were given 2000 cc. of dextran prior to the bleeding time determinations and measurement of blood loss. Patients 18 and 19 had bleeding times prolonged greater than 60 minutes. Patient 18, however, showed no increase in blood loss at operation, while patient 19 had a significant increase in blood loss. Patient 17 did not have bleeding time determinations because his ears were badly burned. He was given 2000 cc. of dextran and his blood loss was not increased.

Patients 14 and 15 were studied in further detail. The bleeding time in patient 15 was not prolonged when he was given one liter of dextran, or when he was given two liters of small-molecular dextran (Table 2). It was significantly prolonged (greater than 60 minutes), however, when he was given 2000 cc. of dextran (Lot No. 13301A); and again after he was given 2000 cc. of large-molecular dextran.

The bleeding times in patient 14 after infusion of 1000 cc. of dextran and after 2000 cc. are shown in Table 3.

Several coagulation studies were performed on patients 14 and 15 (Table

ment of blood loss was discontinued after 20 minutes. The blood-soaked pads were then weighed and the amount of blood loss was determined by the gravimetric method.[5] For increased accuracy, a duplicate blood loss determination was carried out by the colorimetric method.[5]

A celluloid sheet marked in square centimeters was placed over the skin on the Reese tape backing in order to measure the exact amount removed. Immediately after the removal of the skin, the wound was covered with a layer of fine-mesh gauze. Blood loss was calculated in milliliters per 100 square centimeters of removed skin.

Table 1. Blood Loss after Dextran and Albumin*

PATIENT	BLEEDING TIME AFTER DEXTRAN			BLOOD LOSS	
	INITIAL (MIN & SEC)	MOST PROLONGED AT HRS	MOST PROLONGED (MIN. & SEC)	DEXTRAN (CC.)	ALBUMIN (CC.)
1†	5′ 20″	4	9′ 20″	40 0	33 72
2	3′ 30″	1	3′ 50″	58 4	—
3	2′ 45″	4	3′ 55″	13 0	—
4	7′ 03″	4	9′ 0″	57 5	40 4
5	1′ 57″	4	2′ 35″	19 5	18 3
6	2′ 30″	1	2′ 40″	14 5	21 1
7	2′ 55″	1	7′ 38″	45 0	35 0
8	2′ 0″	1	6′ 5″	38 3	11 3
9‡	4′ 6″	12	5′ 35″	36 3	13.9
10	2′ 45″	24	4′ 50″	41 9	42 9
11	1′ 46″	4	3′ 50″	17 5	13 5
12	1′ 56″	24	11′ 7″	25 2	31 5
13	1′ 45″	4	4′ 37″	14 1	13 6
14	2′ 40″	8	24′ 25″	116 0‖	47 8
15	1′ 50″	24	7′ 57″	70 6‖	16 3
16§	2′ 1″	1	7′ 11″	14 1	9 8
17	—	—	—	15 8	15 8
18	4′ 3″	4	60′‖	30 6	30 4
19	3′ 23″	1	60′‖	336 0‖	100 0

10

‡Patients 9 through 15, bleeding time after infusing 1000 cc. of dextran; blood loss after 2000 cc. of dextran and after 2000 cc of albumin

§Patients 16 through 19, bleeding time after infusing 2000 cc. of dextran; blood loss after 2000 cc. of dextran and after 2000 cc of albumin

‖Additional, prolonged bleeding occurred

The above procedure was repeated approximately one week after the first operation, using a 5 per cent serum albumin solution. The skin was removed from a comparable area.

RESULTS

On all patients the red blood count, hemoglobin determination, platelet count, Lee and White coagulation time, prothrombin time, and urinalysis were within normal limits preoperatively.

Blood loss determinations by the colorimetric method paralleled the determinations by the gravimetric method.

Table 4 Coagulation Studies after Infusing Dextran (2000 cc.)

PATIENT	INFUSION	BLEEDING TIME	PLATELET	LEE-WHITE	CLOT RETRACTION	RCB	HEMOGLOBIN	RUMPEL-LEEDE	CLOT LYSIS 24 HRS.
No 14*	Pre-	5′ 35″	347,600	9′	30′	4.4	14.1	Neg.	No
	Post-	21′ 38″	370,000	15′	60′	3.78	11.9	Neg.	No
	After 4 hrs.	60′+	280,000	12′	60′	4.17	12.2	Pos.	No
No 15†	Pre-	3′ 23″	347,600	9′	30′	4.4	14.4	Neg.	No
	Post-	6′ 8″	315,360	8′	90′	4.32	13.6	Neg.	No
	After 4 hrs	60′+	320,000	10′	60′	3.9	11.0	Pos.	No

*Lot No. 13301A Laros, average molecular size 75,000.
†Specialized fractionated C S.C. dextran, average molecular size 91,700

4). The only abnormal finding was a positive Rumpel-Leede test when the bleeding time was most prolonged.

DISCUSSION

Furth and co-workers[2] observed significant bleeding after the infusion of dextran (Lot No. 13301A). The dextran used in this study was from the same lot. To assess better the role of dextran in increased blood loss, control studies with a similar macromolecular substance (5 per cent serum albumin) were performed in all patients. Each patient served as his own control.

There was a significant increase in blood loss after dextran in some patients. The findings suggest that increased bleeding time and increased blood loss may be related to the amount infused and to the molecular size of the dextran. Significant bleeding was observed in three patients, and there was questionable blood loss in one of the 11 patients who received 2000 cc of dextran Studies in patient 15 showed that increased bleeding occurred after infusion of 2000 cc. of large-molecular dextran, but bleeding

Table 3. Bleeding Time after Infusing Dextran (Patient 14)

AMOUNT OF DEXTRAN	PRE-INFUSION	POST-INFUSION	HOURS AFTER INFUSION		
			4	8	24
1000 cc *	2′ 40″	2′ 33″	2′ 15″	24′ 25″	3′ 45″
2000 cc *	5′ 35″	21′ 38″	60′+		

*Lot No 13301A Laros, average molecular size 75,000.

was not increased after infusion of a like amount of small-molecular dextran. It is interesting to note that small-molecular dextran (average molecular size 43,000 and 48,000) was given to the casualties in Korea and no increased bleeding was recognized.[3]

The exact mechanism responsible for increased bleeding after dextran is not clear at this time. Positive Rumpel-Leede tests were seen in patients who exhibited prolonged bleeding This phenomenon was also observed by Adelson.[4] This suggests that patients who bleed after infusion of dextran may have increased capillary fragility

SUMMARY

1 Bleeding times were determined after the infusion of various amounts and molecular sizes of dextran

2 Blood loss from a standardized wound (removal of a drum of skin) was measured after the infusion of like amounts of dextran and 5 per cent serum albumin in 19 patients.

3. There was *no increase in blood loss* in eight patients after the infusion of 1000 cc. of dextran.

4. Blood loss was definite in three and questionable in one of 11 patients who received 2000 cc of dextran

5. Increased bleeding after infusion of dextran seemed to be related to the amount infused and to the molecular size.

6. Results of this preliminary investigation of one lot of one brand of dextran emphasize the urgent need for more extensive studies of all brands

Simple drainage was usually adequate therapy, but wound healing was often prolonged. The most serious infection occurred in the laparotomy wound of patient J.C. His temperature spiked, beginning on the fifth postoperative day. On the seventh postoperative day it was noted that the edges of the incision were reddened, and drainage of the upper wound was instituted. By the eleventh postoperative day the temperature returned to normal and thereafter remained normal. However, gradual separation of the edges of the upper third of the wound with marked undermining of the skin and necrosis of the subcutaneous tissue occurred. The exposed fascia remained intact. This patient received topical bacitracin from the sixteenth to the nineteenth postoperative day and oral chloramphenicol from the seventeenth to the twenty-sixth postoperative day. The photographs (Fig. 1) show the wound on the thirteenth, forty-second, and ninety-seventh



Fig 1 Infected abdominal wound of patient J C *A*, 13th day. *B*, 42nd day. *C*, 97th day.

postoperative days. Although in this instance healing required slightly more than one hundred days, the other six wounds were completely healed in less than thirty days. None of the other wound infections was serious enough to require any medication to supplement drainage, the penicillin and streptomycin having been stopped in the early postoperative period.

Bacteriologic examination revealed that a "plasma clumping positive" hemolytic *Staphylococcus aureus* was present in all seven wounds. In every case the staphylococcus had the same sensitivity pattern (Table 2): resistance to penicillin, streptomycin, Aureomycin, Terramycin, and tetracycline; and sensitivity to chloramphenicol, bacitracin, erythromycin, and magnamy-

to determine if these observations are related to method of manufacture, molecular size, or other related factors. Until such studies are completed, it would appear that continued use of dextran should be encouraged. The likelihood of troublesome bleeding after infusing dextran in amounts for the usual clinical needs is remote.

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AN OUTBREAK OF WOUND INFECTIONS DUE TO ANTIBIOTIC-RESISTANT STAPHYLOCOCCUS AUREUS*

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Surgical wound infections due to antibiotic-resistant strains of *Micrococcus pyogenes* var. *aureus (Staphylococcus aureus)* have become increasingly important since the widespread use of antibiotics.[1-3] This report concerns an epidemic of seven such infections occurring on one surgical service within a thirteen day period.

Table 1. Clinical Data

PATIENT	DATE	OPERATION
E.H.	11-6-52	Left radical mastectomy
T.P.	11-7-52	Cholecystectomy
J.R.	11-8-52	Subtotal thyroidectomy
J.C.	11-8-52	Vagotomy and gastroenterostomy
C.H.	11-11-52	Excision recurrent retroperitoneal carcinoma
J.M.	11-15-52	Splenectomy
A.R.	11-18-52	Stripping of varicose veins

The date and type of operation are presented in Table 1. All patients received penicillin and streptomycin after surgery. All seven wound infections were extensive but involved only the skin and subcutaneous tissue.

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ity pattern as the originally isolated organism almost two years after this incident.

This outbreak is a striking example of a problem which has been recognized since the introduction of antibiotics, that of surgical wound infections caused by antibiotic-resistant organisms.

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THE EFFECT OF TRYPSIN-INDUCED FIBRINOLYSIS ON THE EARLY LATENT PHASE OF WOUND HEALING*

H. DAVID ROACH AND HAROLD LAUFMAN

An increase in the fibrinolytic activity of the circulating blood has been reported in a variety of conditions, such as in carcinoma of the pancreas, liver disease, burn shock, abruptio placentae, amniotic fluid embolism, toxemias, radiotherapy, plasma transfusions, and pre- and postoperatively. The amount of the increase varies from that which can be detected only by sensitive quantitative methods to actual afibrinogenemia.

In the early latent phase of wound healing, a deposition of fibrin bridges the edges of the wound. Laufman and Heller[1] have shown that if anticoagulants have any deleterious effect on wound healing, it is in the first 48 to 72 hours The possibility exists that an increase in fibrinolytic activity of the blood at this time, from any cause, might result in partial or complete lysis of the fibrin bridge and thereby reduce the tensile strength of the wound during this early healing period.

Our experiments were undertaken to determine the effect of trypsin-induced fibrinolysis on the tensile strength of wounds in dogs during the early latent phase of wound healing. Collateral observations were made on the effect of increased fibrinolysis on the initiation of bleeding into a wound.

METHODS

Two hundred thirteen segments from 40 wounds in 24 adult mongrel dogs were tested in these experiments. The skin and subcutaneous tissues over each external jugular vein were incised for a distance of 8 to 12 cm. down to the fascia covering the vein. Careful hemostasis was secured, and the

* From the Department of Surgery, Northwestern University Medical School, Chicago, Illinois This study was supported in part by a grant from The Armour Laboratories, Chicago.

cin. The tetracycline, erythromycin, and magnamycin were tested as they became available The disk method (Tables 2 and 3) was used in determining sensitivity. The tube dilution method gave similar results. The identical sensitivity pattern of the staphylococci suggested a common source for all the infections.

Cultures of the noses and throats of the patients postoperatively contained antibiotic-resistant staphylococci in only three instances. However, staphylo-

Table 2. Sensitivity Pattern of Staphylococci from Patients' Wounds

	ZONE OF INHIBITION AROUND DISK IN MM.						
	R.M.	T.P.	J.B.	J.C.	C.H	J.M	A.R.
Penicillin	0	0	0	0	0	0	0
Streptomycin	0	0	0	0	0	0	0
Tetracycline	0	0	0	0	0	0	0
Aureomycin	0	0	0	0	0	0	0
Terramycin	0	0	0	0	0	0	0
Bacitracin	26	24	22	28	26	19	25
Chloramphenicol	27	32	32	34	32	27	34
Erythromycin	26	28	23	26	27	24	28
Magnamycin	23	20	20	20	20	21	18

cocci having the same pattern of resistance and sensitivity were isolated from the nose of the first assistant surgeon. In contrast, staphylococci isolated from the noses of the surgeon and second assistant were sensitive to all nine antibiotics (Table 3). These three members of the operating team were the only persons who were present at all seven operations, and who cared for all seven patients postoperatively.

The first assistant had been hospitalized and received one gram of

Table 3 Sensitivity Pattern of Staphylococci from Noses of Operating Team

	ZONE OF INHIBITION AROUND DISK IN MM		
	SURGEON	1ST ASST	2ND ASST
Penicillin	27	0	26
Streptomycin	23	0	23
Tetracycline	30	0	24
Aureomycin	28	0	31
Terramycin	28	0	33
Bacitracin	16	29	28
Chloramphenicol	23	28	28
Erythromycin	22	26	22
Magnamycin	19	22	30

Aureomycin orally each day from October 17 to October 30, 1952, for primary atypical pneumonia. He rejoined the surgical team on November 6, 1952, which date coincided with the beginning of this epidemic. His nose and throat cultures prior to the Aureomycin administration had been negative for *Staphylococcus aureus* Staphylococci *having the same antibiotic* sensitivity pattern were cultured not only from the nose but also from the hair, skin, and fingernail scrapings of this individual.

Table 1. Dosage and Rate of Administration of Trypsin

GROUP	DOG NO.	WT. (KG.)	TRYPSIN UNITS PER KG BODY WT.	TIME OF ADM. (MIN.)	PLASMA FIBRINOGEN MG %		CLOTTING TIME WHOLE BLOOD		AUTOLYSIS CLOT OF BLOOD, DRAWN AT TIME WOUND CLOSED
					PRE-INFUSION	POST-INFUSION	PRE-INFUSION	POST-INFUSION	
Wounds closed before afibrinogenemia produced	25	11.3	33,200	300	Not done	0	Normal	Incoag.	No lysis
	29	13.6	73,500	210	505	0	Normal	Incoag.	No lysis
Wounds closed after afibrinogenemia produced	30	11.4	43,800	210	Not done	0	Normal	Incoag	Incoag.
	37	11.4	43,800	210	Not done	0	Normal	Incoag.	Incoag.
	62	13.6	35,000	120	336	0	Normal	Incoag.	Incoag.
	64	10.5	35,000	120	468	0	Normal	Incoag.	Incoag.
	65	12.3	35,000	120	465	0	Normal	Incoag.	Incoag.
Wounds closed after fibrinolysis produced*	52	9.0	19,000	70	280	159	Normal	Increased	Complete
	53	9.5	19,000	70	402	411	Normal	Increased	Slight
	54	9.0	19,000	70	Not done		Normal	Increased	Complete
	55	8.7	19,000	70	Not done		Normal	Increased	Complete
	56	12.3	19,000	70	Not done		Normal	Increased	Complete
	57	9.0	19,000	70	Not done		Normal	Increased	Complete

*Clot of shed blood will undergo complete lysis at 37.5°C. within 24 hours.

wounds were covered with a moist saline sponge until closed approximately one hour later. All wounds were closed in one layer with a continuous 3-0 white silk suture. Drains or dressings were not used. The wounds were observed before and after closure for initiation of hemorrhage from the wound surfaces.

Forty-eight hours later a block of skin and subcutaneous tissue extending 1 cm. on either side of the incision was excised. If there was evidence of infection or of recent escape of more than 50 cc. serum or blood, the specimen was discarded. The excised strips of skin were placed between moist saline sponges until the tensile strength was determined one hour later. A strip of skin was placed on a board and secured at either end with thumb tacks to prevent contraction, but care was exercised not to stretch the specimen. The strip of skin was then divided into segments 1 5 cm. wide, and the sutures were carefully removed.

A modification of the tensiometer used by Wolfer, Farmer, Carroll and Manshardt[2] was used to measure the tensile strength of each wound segment in grams. The column of water necessary to separate the wound edges was weighed and recorded in grams.

Total plasma proteins and fractions, and plasma fibrinogen levels were spot-checked in animals of all series.

Increased fibrinolytic activity was produced in 13 dogs by the injection of trypsin intravenously in various dosages and rates of administration (Table 1) The clot … of fibrinolytic activity of the b … l into three groups. In the first g … he wounds were closed by administration of intravenous trypsin immediately after closure of the wounds. In a second group afibrinogenemia was produced before the wounds were closed by administration of trypsin during surgery. A third group was treated similarly except in the degree of fibrinolytic activity produced, the blood of these dogs was not rendered afibrinogenemic, but fibrinolytic activity was definitely increased.

RESULTS

Control Series (17 wounds in 11 dogs; 78 wound segments) Tensile strength values in this group provided a baseline from which comparisons with figures from the several other series could be made. The mean tensile strength of wound segments in the control group was 182 gm. The tissues adjacent to the line of incision showed an expected amount of edema, moderate staining with blood, and some friability In this series hemorrhage from the suture line did not occur postoperatively up to the time of sacrifice.

Wounds Closed before Afibrinogenemia Produced (4 wounds in 2 dogs, 20 wound segments). There was moderate hemorrhage from the suture line in each anima … were sutured … from one site … It ceased after the circulating blood regained its ability to clot, approximately three hours after the infusion of trypsin was completed. At the time of sacrifice, hematomas and incoagulable blood were found beneath each wound. The amount varied from 15 to 30 cc There was no drainage from the wounds at time of sacrifice and the wound edges were dry. The sub-

Table 2. Summary of Results

	Control Series	Treated Series: Wounds Closed Before Afibrinogenemia Produced	Treated Series: Wounds Closed After Afibrinogenemia Produced	Treated Series: Wounds Closed After Fibrinolysis Produced
Mean tensile strength, Grams	182	172	118	148
Statistically significant difference from control series	—	No	Yes	Yes
Plasma fibrinogen level at time wound closed	Unaltered	Unaltered	0	Decreased
Clotting time of blood at time wound closed	Normal	Normal	Incoagulable	Increased
Hemorrhage from wound after closure	+	+++	++	+ to ++
Reinitiation of hemorrhage from wound before closure			Yes	
Clotting time of blood 4 hours after infusion of trypsin completed	Normal	Prolonged	Prolonged	Normal or slightly prolonged
Evidence of fibrinolysis in blood 4 hours after infusion of trypsin completed (clot lysis test)	—	None	None	None
Evidence of hemorrhage from wound 4 hours after infusion of trypsin completed	—	None	None	None
Clotting time of blood 24 hours after surgery	Normal	Normal	Normal	Normal
Aprothrombinemia	No	Present	Present	Prothrombin time reduced

cutaneous tissues about the wound exhibited some ecchymosis and were considerably more friable than in the control series. The mean tensile strength in this series was 172 gm.

Wounds Closed after Afibrinogenemia Produced (10 wounds in 5 dogs; 56 wound segments) Slight bleeding from the wound appeared immediately before the blood became incoagulable and increased until the wound was closed. There was some bleeding from the incision until resumption of clotting one to two hours after the trypsin infusion was completed. At the time of sacrifice 48 hours later there was no evidence of recent drainage from the wound. Hematomas and collections of incoagulable blood were found beneath the skin in each wound, varying in amount from 10 to 20 cc. Generalized ecchymosis of the tissues adjacent to the wound was seen. These tissues were slightly less friable than those in the previous group. The mean tensile strength of this series was 118 gm.

Wounds Closed after Fibrinolysis Produced (12 wounds in 6 dogs; 59 wound segments). Oozing occurred from the wounds at time of closure, although no additional hemostasis was required, but the oozing ceased spontaneously after approximately one hour. With one exception no hematomas were present under the wounds, although a collection of incoagulable blood varying from 5 to 10 cc. was found The tissues adjacent to the wound were moderately edematous and friable Mean tensile strength in this series was 148 gm.

A summary of results is shown in Table 2.

STATISTICAL ANALYSIS*

Statistical comparison was made between tensile strengths of 1.5 cm wound segments in the control group and those in each of the other three groups, by the method of pooled sums of squares.

Tensile strengths of wounds closed before afibrinogenemia was produced showed no significant difference from the tensile strengths of wounds in control animals

On the other hand, the tensile strengths of wounds closed after production of either fibrinolysis or afibrinogenemia were significantly less than tensile strengths of wounds in control animals, the chance occurrence of such a difference being less than 1 in 100.

DISCUSSION

The degree of secondary hemorrhage from the suture line after closure of the wounds appeared to be directly related to the increase in the fibrinolytic activity produced, and therefore proportional to the trypsin dosage and rate of administration. This may also hold true for the increased edema and friability of the subcutaneous tissues adjacent to the wound. It has been suggested that the secondary hemorrhage that occurs after using anticoagulants postoperatively may be due to stimulation of some internal mechanism that will cause digestion of the clots in small arterioles and venules.[4] Our experiments on the effect of increased fibrinolysis on intravascular blood clots do not support this concept in the case of trypsin-induced fibrinolysis.[3, 5]

The presence of incoagulable blood beneath the incision in animals in-

* The authors are indebted to Harry M Richter, Jr, M D, who carried out the statistical analysis of the data.

EXPERIMENTAL PRINCIPLES OF REPAIR OF COMPLETE TRACHEAL DEFECTS*

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The desirability of being able to excise a segment of the tracheobronchial tree and effectively reconstruct the defect so created is apparent from the number of papers which have appeared in recent years dealing with both the experimental and clinical aspects of the problem. The variety of methods suggested in these papers attests further to the lack of any one completely satisfactory scheme. Our own experience with reconstruction following sleeve resection of the cervical trachea in 75 dogs has shown several principles which seem essential if success is to be attained.

METHODS OF RECONSTRUCTION

The various methods of reconstruction which were studied were:

- I. Tube of stainless steel mesh
  - A. Mesh alone
  - B. Mesh covered
    - 1. Fascia lata
    - 2. Aortic homograft
    - 3. Gelatin sheet
    - 4. Split-thickness skin graft
- II. Spiral wire covered with split skin graft
- III. Polyethylene tube
  - A. Plain tube
  - B. Covered by strips of periosteum

In all cases a circumferential resection of from 5 to 10 cm. of cervical trachea was carried out. In the first 40 animals the tracheal ends were placed inside the prosthesis. This was done in the hope that epithelization would proceed from the cut tracheal ends and ultimately bridge the entire defect. The wire mesh, a porous material, was employed to make available to the epithelium an underlying bed of granulation tissue.

All of the techniques using wire mesh either alone or covered by the various materials listed can be considered in a single group. Results in all were monotonously unsuccessful. Epithelization from the cut ends in no case extended for more than a few millimeters and consistently ended in a bud of granulation tissue which usually caused death from airway obstruction after a few weeks (Fig. 1). In the animals with the mesh covered by fascia, aortic homograft, or gelatin, the results were the same with the added complications of foreign body reaction, infection and in some cases formation of fistulous tracts to the skin. We were unable to obtain satisfactory results in the experiments using split skin grafts over the mesh, the grafts never growing and the end results being similar to the others. This is scarcely surprising when one considers the contaminated area and the mobility of the bed.

In the next series of experiments polyethylene tubing was employed as

* From the Department of Surgery and the Bonfils Tumor Clinic, University of Colorado School of Medicine, Denver, Colorado. Supported by a grant from the American Cancer Society and the Damon Runyon Fund.

fused with trypsin after closure of the wound may be due, perhaps, to the mobilization or autolysis of small clots. Trypsin infusion experiments[5] have shown that after afibrinogenemia has been produced, the values of clotting constituents of the blood will have returned to clotting levels. However, there may be a lag period in which autolysis of a clot of shed blood will occur. The difference in quantities of incoagulable blood and size of hematomas present in trypsin-infused animals may perhaps be explained by differences in trypsin dosage and by variations in mechanical hemostasis.

The presence of incoagulable blood under the skin in animals infused with trypsin before closure of the wound may be explained by incoagulable blood seeping into the wound after closure, in addition to some autolysis of shed blood clots during the period of increased fibrinolysis. The absence of hematomas would suggest that the amount of increased fibrinolysis in this group caused secondary hemorrhage to occur only during the time that fibrinolysis was present.

We have shown in previous experiments[5] that a trypsin-induced increase in fibrinolytic activity in the circulating blood does not cause the lysis of a pre-existing thrombus or embolus. However, we found that fibrinolytic activity sufficient to cause autolysis of a clot of shed blood can cause autolysis of an intravascular clot, provided such increased fibrinolytic activity is produced prior to clot formation. These observations are in agreement with the results of the present experiments.

SUMMARY AND CONCLUSIONS

1. The tensile strength of wounds in the early latent phase of wound healing was measured in a series of control dogs and in dogs in which increased fibrinolytic activity of the blood was produced by the intravenous infusion of trypsin.

2 The tensile strength of wounds closed before increased fibrinolytic activity was produced did not differ significantly from that of the controls.

3. The tensile strength of wounds closed after the production of increased fibrinolytic activity was significantly less than that of the controls; the amount appeared to be proportional to the amount and duration of the increased fibrinolytic activity.

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with interrupted Nos. 30 and 32 steel wire. The stitches were placed through the prosthesis, into the lumen and brought out through the wall of the trachea, incorporating two or three cartilaginous rings. Four to six of these sutures were used on each end. In several animals the tubes became dislodged at one or both ends, either because the suture material broke or because the sutures cut through the trachea. In all instances this was a lethal event. It is apparent that an absolutely dependable method of holding the tube in its proper position is essential.

SIX COMMON CAUSES OF FAILURE IN TRACHEAL REPAIR



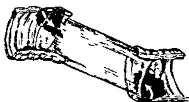
1. GRANULATIONS WHICH FORM ON THE CUT EDGES OF TRACHEA NARROW LUMEN.



2. DISPROPORTION BETWEEN TUBE AND TRACHEA PERMITS INGRESS OF GRANULATIONS.



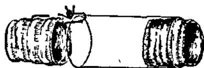
3 PERMEABLE MESH PERMITS THE GRANULATIONS TO GROW THROUGH WALL.



4 TUBE TOO LONG PRODUCING GRANULATION ULCER IN TRACHEAL MUCOSA



5. SUTURE CUTS THROUGH TRACHEAL RING CAUSING BREACH IN CONTINUITY



6. SUTURE BREAKS CAUSING BREACH IN CONTINUITY

Fig. 3

It had been our hope, and that of most other workers in this field, that the tubular prosthesis placed between the severed tracheal ends might be a temporary thing, with removal of this foreign body after a period of weeks or months. This question was answered for us by the spontaneous dislodgment of the tube in several animals. In no case was there evidence of regeneration of surrounding tissues to form a rigid and permanent airway.

In an attempt to prevent collapse of the airway when the polyethylene tube was removed, strips of periosteum taken from the animals' ribs were used as free grafts placed about the outside of the tube. As was true when

the prosthesis. This material was chosen since it is well tolerated by animal tissues and, as a solid material, will not allow granulation tissue to grow through its walls. The technique of insertion was the same, with the cut tracheal ends placed inside the ends of the tubing. Although the period of survival was somewhat longer in this group, ultimately respiratory obstruction from granulations arising from the cut tracheal ends was fatal.

The remaining experiments were carried out using polyethylene tubes as



Fig. 1. Section shows epithelium ending in a bud of granulation tissue

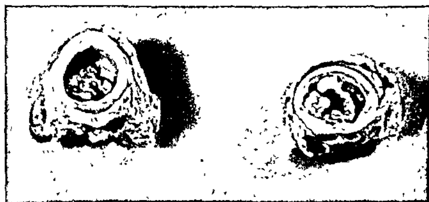


Fig. 2. Dog 40. Complete resection. Death 8 days postoperatively from stenosis.

the prostheses placed *within* the lumen of the cut tracheal ends. An improvement in results was soon apparent. Obstruction of the airway from granulation tissue occurred less often, and in those instances was the result of either a disproportion between the size of the trachea and the size of the tube, allowing ingress of inflammatory tissue, or ulceration of the bronchial mucosa from an overlarge tube. Both of these are avoidable technical errors.

As more experience was gained with this technique a new difficulty presented itself. Fixation of the tube to the tracheal ends had been carried out

PRESENT TECHNIQUE OF RECONSTRUCTION

Two methods of fixation of the polyethylene tube in the trachea have given reasonably consistent satisfactory results. The first of these consists in suturing the tube to adjacent tendinous and soft tissue structures with No. 28 stainless steel wire, since simply sewing the tube to the cut tracheal ends was frequently found to result in the sutures cutting through and allowing the tube to become dislodged. Of 10 dogs having this type of reconstruction, 9 were followed for periods up to one year and are doing well. The one dog that died developed obstruction from granulation tissue [illegible] tracheal mucosa. [illegible] principle devised [illegible] nylon ring on the inner side of which are a number of teeth. The ring is placed on the outside of the trachea after a polyethylene tube has been inserted within the cut ends. The teeth hold the tracheal wall firmly against the tube, and small flanges on either end of the tube prevent it from slipping. Of 8 dogs having tracheal reconstruction by this method there has been no fatality attributable to the method. Two dogs died during an epidemic of distemper and at autopsy had findings consistent with this diagnosis.

A permanent tubular prosthesis is a necessary part of any scheme that will successfully correct a complete circumferential defect of the trachea. Polyethylene, an inexpensive, readily available, pliable material which is well tolerated by animal tissue, was found to make a satisfactory tube. As has been pointed out, if the cut tracheal ends are placed within the tube, obstruction of the airway by granulation tissue will consistently result. Thus, the prosthesis must be placed inside the tracheal lumen, and the tube must be firmly anchored so it will in fact be permanent.

Despite the theoretical objections, the expulsion of respiratory secretions across the polyethylene stent has not been a major problem. The dogs have maintained their ability to cough and pulmonary sepsis has occurred only once since the present reconstructive techniques have been employed.

CONCLUSIONS

1. Respiratory obstruction from granulation tissue is the main obstacle to successful repair of sleeve defects of the cervical trachea.
2. This obstacle is best overcome by the use of a permanent prosthesis of proper size inserted within the lumen of the tracheal ends.
3. Polyethylene tubing has been the most satisfactory prosthesis.

REFERENCES

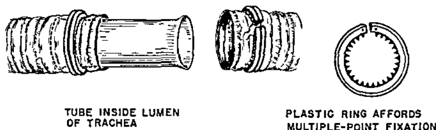
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2. Craig, Robert, Holmes, George, and Shabert, E.: Tracheal resection and replacement with prosthesis J Thoracic Surg, 25:384–396, 1953.
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skin grafts were used, the periosteum failed to grow. A few animals showed x-ray evidence of some calcification in the area, but in no case was there a supporting skeleton sufficient to maintain a rigid airway. In addition, a significant number of animals with periosteal grafts developed abscesses and cutaneous sinuses.

REQUIREMENTS FOR SUCCESSFUL TRACHEAL RECONSTRUCTION

From the experiments which have been discussed, several principles essential for satisfactory reconstruction of complete sleeve defects of the trachea are obvious.

A METHOD OF TUBE FIXATION UTILIZING PRINCIPLE OF HUFNAGEL'S AORTIC VALVE RING



ALTERNATE METHOD USING POLYETHYLENE TUBE AND WIRE SUTURES (LONGITUDINAL SECTION)

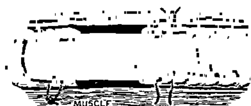


Fig. 4

1. The prosthetic tube must be permanent There is no evidence to suggest that the mediastinum has any mystical property of tracheal regeneration, contrary to earlier reports.[1] We agree with Craig that "true tracheal regeneration does not occur after sleeve resection "[2]

2. The bridging tube must be placed inside the lumen of the cut tracheal ends. If this is not done, respiratory obstruction from granulation tissue can be expected.

3. The tube should be impervious to granulations growing in through the walls.

4. The tube must properly fit the severed tracheal ends

5 Fixation of the prosthesis in position must be dependable.

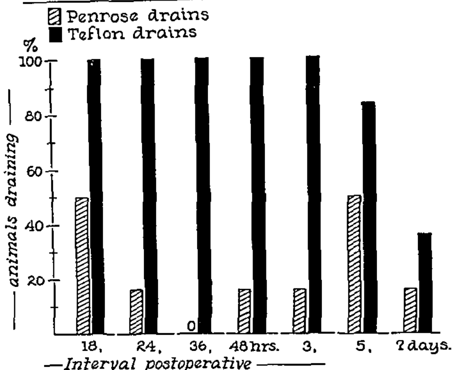

COMPARISON OF DRAINAGE PROPERTIES
Penrose drains
Teflon drains
%
100
80
60
40
20
— animals draining —
0
18,
24,
36,
48 hrs.
3,
5,
7 days.
—Interval postoperative —

Fig. 1.

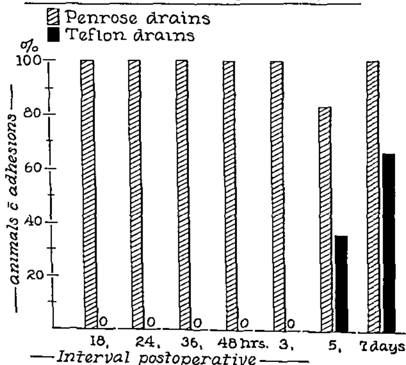

COMPARISON OF ADHESION FORMATION
Penrose drains
Teflon drains
%
100
80
60
40
20
—animals c̄ adhesions—
0
0
0
0
0
18,
24,
36,
48 hrs.
3,
5,
7 days
—Interval postoperative —

Fig. 2

STUDIES OF A DRAIN OF A NON-REACTIVE PLASTIC, TEFLON*

FREDERICK W. PITTS AND JONATHAN E. RHOADS

The use of a non-reactive plastic, Teflon,† as a drain for the peritoneal cavity was studied in rats. *Teflon is the trade name for polytetrafluorethylene*, a plastic having the properties of little or no tissue reactivity[1] and of minimal adhesiveness. These properties suggested that drains of this substance might permit the escape of drainage more freely for a longer period of time and cause less adhesion formation due to irritant properties of the drain itself.

METHOD

A fluted Teflon drain was compared with a rubber Penrose drain with a gauze filler (cigarette drain) as the control. The fluted Teflon drain was made by folding a piece of Teflon sheet 1 inch wide four times with the folds parallel to the long axis. The drains were all 1½ inches long. The Penrose drains were 5/16 inch in diameter. The Teflon used was 0.003 inch in thickness. The drains were washed with ethyl alcohol to remove any extraneous material and then, after the gauze fillers inserted in the Penrose drains, autoclaved. The operative procedure to install the drains was done under ether anesthesia. Sterile technique was used with the exception that gloves were not worn. The peritoneal cavity was opened through an upper abdominal mid-line incision and the drains placed through a stab wound in the right side. The peritoneum with overlying muscle was then closed with a continuous suture of black silk. The drain was secured with a stitch of black silk passing twice through it and the skin. The main skin incision was then closed with a continuous suture of black silk. The drain site was dressed with gauze and adhesive tape to prevent the animals from removing the drain. A total of 96 Wistar white rats each weighing approximately 150 gm was used.

The drainage qualities of the various drains were tested by the injection of 5 cc. of an aqueous solution of methylene blue intraperitoneally at a site distant from the drain and then noting the appearance of the dye at the drain site. The criterion of drainage was the appearance of any dye, no matter how little, at the drainage site. The animals were autopsied 15 to 30 minutes after injection of the dye This was to observe the causation of any blockage and to note the presence of adhesions to the drain and drainage site. The criterion of adhesion formation was the presence of any adhesion affixed to the drain or any abdominal viscus that did not readily slide away incident to the folding back of the abdominal wall when viewing the contents.

RESULTS

Figures 1 and 2 present the results graphically. Various aspects of the results not demonstrable graphically should be mentioned: (1) Drainage

* , University of

†

PLASTIC MODELS OF THE TRACHEOBRONCHIAL TREE*

CHARLES V. MECKSTROTH AND KARL P. KLASSEN

One of the greatest problems in teaching pulmonary surgery to the intern and resident staff is the complex anatomy of the tracheobronchial tree and its segments. Formalin-fixed cadaver tissue is of limited value and time is generally not available during routine postmortem examination for this detailed and painstaking study. Because of this, most thoracic surgeons learn pulmonary anatomy by roentgenographic study and in the operating room. For these reasons the authors have investigated reconstruction of the human tracheobronchial tree with plastic to produce three-dimensional models for teaching purposes.

Early in the work a certain plastic was used which was satisfactory as far as technique was concerned but displayed marked brittleness upon completion of the cast. A search was then made for a plastic of controllable viscosity which would produce a flexible cast to allow the necessary close handling. After considerable investigation† a suitable plastic was found and the details of processing have now been perfected.

The technique is simple and can be performed in most laboratories. The entire process from a fresh lung to finished product takes approximately two weeks, although the time can be lessened by various shortcuts. The cost of injecting a human lung, including the pulmonary artery and vein, is approximately $5.00. The finished product will not chip or break, and because of its flexibility can withstand much handling.

METHOD

Fresh human lungs including a long trachea, intact pulmonary artery, and intact left atrium are washed externally with running water and with manual pressure the blood is pressed from the artery and veins of both lungs. Care is taken to prevent water from entering the trachea. Biopsy of various peripheral areas can be taken for histologic examination. If aspiration of mucus took place before expiration the tracheobronchial tree should be aspirated with a small catheter. Rubber tubing 1 inch O.D., ¾ inch I.D. and 6 inches in length is used to intubate separately the left atrium and main pulmonary artery. A purse-string suture of 0 silk is placed through the atrium and tubing to "gather" the vessels around the tubing and make an airtight seal. The trachea should be similarly intubated with a smaller rubber tubing of ¾ inch O.D. If only the tracheobronchial tree is desired, the process is exceedingly simple. The lung is then inflated with air from the airline in the laboratory or a positive pressure pump. All sutures being airtight, the lung as well as the pulmonary vessels will be inflated into a normal, distended position. Overdistention must be prevented by regulating the flow of air with a safety valve. Even if several small biopsies of the lung have been taken the lung will still inflate. Areas of atelectasis are

* From the Division of Thoracic Surgery, Department of Surgery, The Ohio State University Health Center, Columbus. This study was aided by the George Morris Curtis Research Fund.

† Aided by the staff of the Plastics Division of Battelle Memorial Institute.

observed with the Teflon drains was in all instances immediate and profuse, while drainage which occurred from the Penrose drains was slight and delayed, and (2) Teflon drains that showed no drainage and adhesions at the 5 and 7 day intervals had gross infection of the drainage site with the remainder of the abdomen clear, while animals showing drainage revealed no indication of infection.

CONCLUSIONS

Results of this experiment lead to the conclusion that Teflon drains, which are made of a non-reactive substance, remain functional significantly longer than do Penrose drains, which evoke a peritoneal reaction. It may also be concluded that fewer adhesions formed in the rat secondary to a drain of this type than to a Penrose drain.

Additional work in animals is still in progress. The condition of the peritoneal cavity following removal of the drain on the seventh day has been studied in a small group of six animals using the Teflon drain and six control animals using rubber dam drains. Results with this small number have shown no adhesions secondary to the use of the Teflon drain in four of the six as observed three weeks after its removal. The control group has had a 100 per cent incidence of usually dense adhesions after a comparable period of time.

Teflon drains are also being used on a small scale clinically. They have been used in the following operations: 6 cholecystectomies, 2 gastrectomies, 5 thyroidectomies, 1 bowel resection, and 1 cholecystojejunostomy. Although it is difficult objectively to evaluate them clinically, they have in all instances seemed at least comparable to the existing Penrose drains now being used. The favorable results in animals suggest that they may offer advantages.

SUMMARY

1 As studied in the rat, Teflon drains drain longer and cause less adhesion formation than rubber Penrose drains

2 Early work concerning removal of drains shows fewer adhesions several weeks after the removal of a Teflon drain than a Penrose drain.

3. Early clinical work has suggested that Teflon drains are at least adequate substitutes for Penrose drains

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will flow faster in this larger rubber tubing and the definitive trachea is of normal caliber.

Tight fitting funnels are attached to the rubber tubing and the pulmonary artery is poured first, the pulmonary vein second and the trachea last. The specimen with the tubing and funnel still attached is taken to a fluoroscopic [illegible] ge of manual [illegible] much plastic [illegible] a and pulmo- [illegible] by a burette [illegible] clamp attached to the rubber tubing of the pulmonary artery. The lung is then wrapped in aluminum foil to prevent scorching and is placed in a baking oven at 310°F. (158°C.) for about seven hours. It is best if the oven

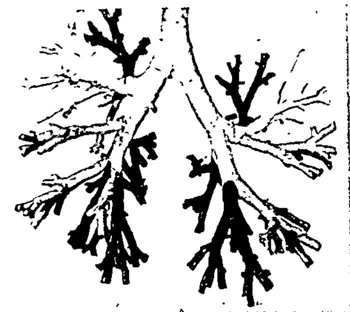


Fig. 2. Trimmed cast of tracheobronchial tree with various segments painted separately.

is vented, as some odor is produced. The lung should not be handled while hot.

Upon cooling, the rubber tubing of the pulmonary artery is cut short and the remaining rubber tubing is removed with scissors. The entire specimen is placed in a 3 gallon container with a burette clamp applied to the solidified trachea from a ringstand placed under the jar. A solution of pepsin, 0.5 per cent in HCl, 0.2 per cent by volume, is then used for digestion.* The container is placed in a sink and is kept warm by circulating water at approximately 120°F. With daily agitation and replenishment of the pepsin solution the process of digestion takes about five to seven days, depending upon the size of the specimen. The cartilages of the tracheobronchial tree will require manual removal with scissors and clamp. When digestion is complete, the residual tissue is picked off and the specimen is cleaned by washing with hot soapy water. The cast can then be trimmed as desired and

* Used at the suggestion of Dr. Clayton S. Smith of the Department of Physiological Chemistry, Ohio State University Medical School.

removed by digital massage. After seventy-two hours of drying, or less if a drying chamber with heat lamps is available, the specimen is ready for plastic instillation. The lung must be completely dry or it will collapse when put in the oven for polymerization.

The proportion of approximately 50 per cent plasticizer* to 100 per cent resin* has given the most satisfactory result and has prevented troublesome alveolar filling Should this be too thick a mixture, 55 or 60 per cent plasticizer can be used. Expendable material for mixing consists of paper cups and wood paint stirrers. In order of mixing, the weights for any one of the three compartments of an average human tracheobronchial tree are as follows: plasticizer, 75 grams (72 cc.); liquid heat stabilizer,† 3 cc.; powdered heat stabilizer, 4.5 grams; light stabilizer, 3 grams; color,‡ 1 gram for red and blue, 3 grams for white (Fig. 1). After these materials are mixed, 125

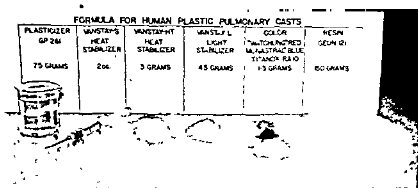


Fig. 1. Materials used for plastic casts in order from left to right. Three separate "batches" are required if artery and vein are to be injected also.

to 150 grams of resin are slowly incorporated. At first the mixture will be quite thin, but after adequate resin has been added, it assumes the viscosity for the usual instillation, that of thick molasses. If the mixture is too thick, more plasticizer may be added.

The rubber tubing in the left atrium and pulmonary artery is left intact but the rubber tubing inside the trachea is carefully removed by cutting the silk sutures. ..
the trachea,
Additional ta
to the tubing to prevent separation of the tube from the trachea. The plastic

* Plasticizer, GP 261—$6 00 per gal.
Geon resin—212—$8 85 per 15 lb.
Obtainable from the B. F. Goodrich Company, Rose Building, 2060 East 9th Street, Cleveland.

† Stabilizers.
Liquid Vanstay S—.65 per lb.
Heat Vanstay HT—.92 per lb.
Light Vanstay L—.35 per lb.
Available from R. T. Vanderbilt Co., 230 Park Avenue, New York 17, New York.

‡ Colors Samples used, prices unknown
White: Titanex—RA50 — Titanium Pigment Corp., New York 6, New York.
Red: "Watchung" Red — E. I duPont DeNemours & Co., Pigment Department, Wilmington, Delaware.
Blue: "Monastral" Blue

DISCUSSION

It is recommended that more easily available material such as dog or rabbit lungs be used prior to work on human lungs. For average-sized dog lungs, the amount of material can be cut in half. If a hood is available, digestion with muriatic acid may be preferable to pepsin since it is less time-consuming and probably less expensive. Fluoroscopic control of the extent of the cast is not mandatory but is desirable, especially on first attempts. By this technique the extent of filling can be limited to the bronchioles and inadequate filling is also prevented. If desired, alveolar filling can be accomplished by using a more viscous mixture and by applying more pressure to the rubber tubing during the pouring procedure. If entrapped air bubbles become a problem, the periphery of the dried specimen can be sanded mechanically to remove the outer ⅛ inch to allow for their escape during the pouring process. The finished casts are shown in Figures 2 and 3, while a general outline of the procedure is shown in Figure 4.

CONCLUSIONS

A practical and inexpensive method of producing casts of the human tracheobronchial tree and vascular tree by instillation of a flexible and durable plastic has been described. We have found these models to be of great value in teaching pulmonary anatomy to medical students, interns and residents. In addition, these casts are extremely helpful to anatomy instructors and physicians dealing with chest diseases.

either painted with plastic paint* (bronchial segments) or sprayed with an acrylic spray.† Stainless steel wire is used to hold the pulmonary artery and vein to the bronchus. A wood stand is fashioned to support the case.

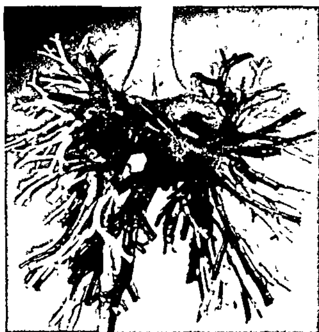


Fig. 3 Trimmed cast of tracheobronchial tree including pulmonary artery and vein.

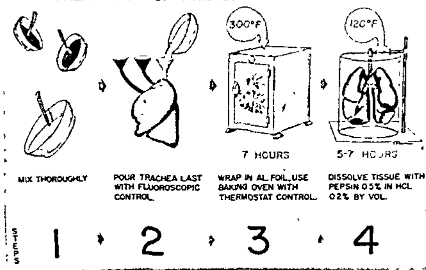


Fig. 4. Diagram of steps required for plastic instillation.

* PLA Paint for Plastics, Testor Chemical Co., Rockford, Illinois

† Krylon Spray. Krylon, Inc., Philadelphia, Pa., $1.95 per pint.

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AUTHOR INDEX

SUBJECT INDEX